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FINAL STAGE I REPORT
DECEMBER 31, 1987
VOLUME 3 of 3

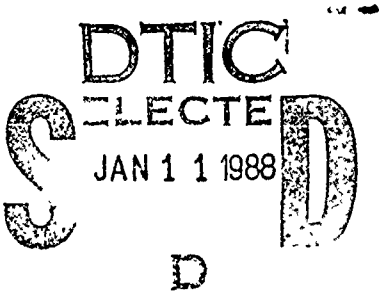
L2

Final Stage I Report
To The US Army Medical Material Development Activity (USAMMDA)

December 30, 1987

Transdermal Drug Delivery System

Contract No. DAMD 17-85-C-5213



Submitted by: Riker Laboratories, Inc.
3M Center, Bldg. 270-4S-02
St. Paul, MN 55144

Maria J. Westfall 12/18/87
Maria J. Westfall Date
Program Manager

W. G. Meredith 12/13/87
W. George Meredith Date
Vice President,
Riker Laboratories, Inc.

Contractor's Report Number
9021000800-009

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CONTRACTOR

3M Company
3M Center, Building 270-4S-02
Riker Laboratories, Inc.
St. Paul, Minnesota 55144-1000

PRINCIPAL INVESTIGATOR

Amit K. Mitra, Ph.D.

PROGRAM MANAGER

Maria J. Westfall, P.E.

OVERALL TABLE OF CONTENTS

TABLE OF CONTENTS
VOLUME 3 OF 3

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In Request for Proposal (RFP) Number DAMD17-84-R-0078, the US Army had requested proposals for a four-stage program aimed at the development, optimization, and production of a transdermal drug delivery system (TDDS) for the drug, pyridostigmine bromide. Stage I of the work had the objective to explore alternative TDDS concepts and In response to that request, 3M's pharmaceutical subsidiary, Riker Laboratories, Inc., proposed the simultaneous exploration of three design approaches; the drug incorporated into 3M proprietary hypoallergenic adhesives, the drug incorporated into a gel diffusion matrix, and the drug delivered from an iontophoretic system.

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19. ABSTRACT

A Minnesota Firm, Medtronic, Inc., was utilized as a subcontractor for the development of an iontophoretic system. During a five-month feasibility study, Medtronic, Inc. provided and tested an iontophoretic system. Preliminary in vitro and in vivo results indicated that pyridostigmine bromide could be delivered by this approach.

A series of 3M hypoallergenic adhesives was formulated with pyridostigmine bromide. In vitro skin penetration of pyridostigmine bromide from nonpolar adhesive based systems was very low, and polar adhesive based systems did not have acceptable physical properties for a pressure sensitive adhesive.

A wide range of components were incorporated into polyvinyl alcohol based gel diffusion matrices. The formulations were screened both in vitro and in vivo and they met initial delivery requirements.

Dermal reactions (i.e. erythema and edema) were observed in rabbits treated with polyvinyl alcohol based formulations. Numerous guinea pig sensitization and repeat dermal irritation studies were conducted on the drug alone, and the active systems. Sensitization and irritation were obserbed with the drug alone, and with the total transdermal system. Irritation and the incidence of sensitization was greater with the transdermal delivery system than with the drug alone. There was considerable variability in the severity of irritation. The dermal reactions increased the penetration of pyridostigmine bromide, which resulted in systemic toxicity. It was evident that the formulations being tested could not be successfully tested in subchronic toxicity studies or in human clinical trials because of variable and unpredictable transdermal drug delivery.

Final technical efforts focused on exploring the concept of a rate-limiting membrane, a membrane that could theoretically limit the amount of drug delivered to the skin and thus overcome the variability in delivery due to unpredictable skin irritation. Although commercially available materials were found that could indeed limit the amount of drug released from the systems, no system was found that released the drug at a rate adequate for the intended use.

Because of the inherent variability in irritation response from pyridostigmine bromide and the subsequent variability in acetylcholinestrase inhibition levels, a trandsermal system could not be developed that resulted in the targeted acetylcholinesterase inhibition levels. Although additional work could potentially result in an effective product, the US Army made a decision to suspend contract funding effective September 2, 1987.

Pathology and Toxicology
Riker Laboratories, Inc.

Building 270-3S-05, 3M Center
St. Paul, Minnesota 55144-1000

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3M

SUMMARY OF SAFETY EVALUATION STUDIES
CONDUCTED IN SUPPORT OF THE
PYRIDOSTIGMINE BROMIDE TRANSDERMAL DEVELOPMENT PROGRAM

AUTHOR:

J. Allen 12-14-87

James L. Allen, Ph.D. Date
Diplomate, A.B.T.
Research Toxicologist

APPROVAL:

S. V. Elrod 12-14-87

Steve V. Elrod, Ph.D. Date
Diplomate, A.B.T.
Manager, Pathology/Toxicology
and Laboratory Animal Medicine

I. INTRODUCTION

The safety evaluation of transdermal pyridostigmine bromide (S-26741) consisted of extensive surveys of the literature and in vivo and in vitro safety studies. A list of the studies conducted is presented in Appendix A. The literature reviews were done for pyridostigmine bromide and the excipients in the transdermal formulation. Over 30 studies were conducted to support the development of a transdermal pyridostigmine formulation. These studies evaluated the acute toxicity, dermal irritation, and sensitization from S-26741 alone and in a transdermal formulation. These studies were done to support selection of a transdermal formulation for further development.

There also was a significant effort expended planning the IND subchronic animal toxicity studies. This effort included contacting contract laboratories, drafting protocols, and reviewing the scientific literature regarding the potential of S-26741 to interfere with functioning of the neuromuscular junction. Draft protocols were prepared to characterize the effect of S-26741 on the neuromuscular junction and determine the relationship of this effect to dose.

II. ACUTE SYSTEMIC TOXICITY STUDIES

Acute systemic toxicity studies were conducted in rats, rabbits, and dogs. S-26741 was administered orally in rats and dogs and dermally in rabbits. Approximate LD50s or minimum toxic doses are presented below.

Table 1
Acute Toxicity of S-26741

Species	Route	LD50 (mg/kg)
Rat	PO	52
Rabbit	TOP	80
Dog	PO	1-10[a]

[a] minimum toxic dose

Drug-related clinical signs in the rat and dog were generally related to effects on the central nervous system (eg, convulsions, hypoactivity). The only drug-related clinical sign in the rabbit study was an increase in salivation. There were no obvious treatment-related gross abnormalities observed during necropsy.

III. IRRITATION STUDIES

Primary skin irritation studies were conducted with many different formulations of S-26741. The formulations tested and irritation rating are presented in Table 2. Several excipients were screened for their dermal irritation potential. Solutions of sodium lauryl sulfate (0.33%) docusate sodium (0.35%) and potassium laurate (1%) were tested and found not irritating to rabbit skin. Formulations of 50% S-26741 in a solution, microporous membrane, or PVA gel with various penetration enhancers generally caused no, minimal, or slight irritation in albino rabbits.

Cumulative dermal irritation studies were also conducted in rabbits by treating rabbits daily for seven consecutive days with 30% S-26741 in a hydroxypropylmethylcellulose (HPMC) gel containing either 0.198% sodium lauryl sulfate or 0.21% docusate sodium. A HPMC gel containing 50% S-26741 alone was also tested for its potential to cause cumulative irritation. There was individual variability in the irritant responses and also toxicity including death, probably as a result of increased transdermal delivery of S-26741 through compromised (irritated) skin. In general, the mean irritation scores of surviving animals progressively increased during the study. Therefore, there was evidence of cumulative irritation with each formulation tested.

Dermal irritation studies were also conducted in albino guinea pigs wherein the S-26741 HPMC gel formulations were administered three times per week for three weeks. The dosing regimen was similar to that used in the guinea pig sensitization studies and was used to evaluate dermal irritation resulting from this dosing regimen. The irritation data from this dosing regimen was used to properly evaluate any dermal reactions observed in the sensitization studies.

IV. SENSITIZATION STUDIES

Guinea pig sensitization studies were conducted with S-26741, formulation excipients and combinations of both.

S-26741 (neat chemical) and a 50% S-26741/0.33% sodium lauryl sulfate formulation were tested using the method of Magnusson-Kligman and no positive responses were observed; however, the doses tested were very low because of drug-related toxicity at higher doses. Thus, the results of this study may have been influenced by low systemic exposure to S-26741 which was insufficient to induce an immunologic response.

In view of the findings observed in a Yorkshire Cross Swine pilot metabolism study, a sensitization study was conducted in two swine using a modified Buehler technique. The animals were induced with a microporous membrane patch containing 50% S-26741 and 0.33% sodium lauryl sulfate in a gel material. The study design required nine topical induction doses, but gross irritation at the application site (and the appearance of clinical signs of toxicity) limited the induction phase to six doses. Dermal reactions (ie, sensitization) were observed in both animals challenged at naive sites with S-26741/sodium lauryl sulfate and S-26741 alone. Given this limited data from only two animals (and no control animals), the S-26741/sodium lauryl sulfate microporous membrane formulation appeared to have a sensitization potential.

A series of guinea pig sensitization studies were conducted to more clearly define the sensitization potential of S-26741 formulations. Formulations tested were HPMC gels containing; 50% S-26741 alone and in combination with 0.33% sodium lauryl sulfate; and 30% S-26741 with 0.198% sodium lauryl sulfate or 0.21% docusate sodium. A modified Split Adjuvant Technique was employed. Briefly, this technique is a modification of the standard test used to determine the sensitization potential of topically-applied formulated materials. This technique utilizes Freund's Complete Adjuvant during the induction phase to render the guinea pig more susceptible to sensitization. The studies with S-26741 formulations were designed to identify the sensitization potential of S-26741 alone or in combination with the surfactants (see Table 3). The induction phase was terminated prematurely (after 6 doses) because of gross dermal irritation and severe toxicity (including death) in some animals. Four of nine

animals induced with 50% S-26741 alone had positive responses (erythema and edema) to a challenge dose with 50% S-26741 (Table 4). Animals induced with formulations containing 30 or 50% S-26741 with sodium lauryl sulfate or sodium docusate had a higher incidence of positive responses to the challenge dose than animals induced and challenged with S-26741 alone. Thus, these studies indicated that S-26741 alone or in the topical formulations is a sensitizer in animals. This finding is supported by recent reports of contact sensitization from structurally related compounds (ie, quaternary ammonium compounds).[1] This information and the data from the guinea pig studies suggest the dermal reactions observed in swine may have been a sensitization reaction.

V. MISCELLANEOUS STUDIES

In vitro cytotoxicity assays were conducted with microporous membranes used in the S-26741 formulations. L-929 mouse fibroblasts were exposed to various microporous membranes and subsequently evaluated for extent of lysis. This information was used to select membranes for further development work.

VI. OVERALL SUMMARY OF SAFETY EVALUATION STUDIES

Acute toxicity studies were conducted with S-26741 in dogs, rabbits and rats. LD50s and minimum toxic doses were comparable with those reported for this drug. Clinical signs of toxicity were consistent with the cholinergic activity of S-26741. No obvious treatment-related gross abnormalities were observed during necropsy.

Primary skin irritation studies in rabbits indicated that 30 or 50% S-26741 formulations generally caused no, minimal, or slight irritation after a 24-hour application.

The potential for cumulative dermal irritation was evaluated in rabbits by applying gel formulations of S-26741 daily for seven consecutive days. Severe toxicity including death was observed as the study progressed. Although the number of animals surviving to the end of the treatment period was small, the mean irritation scores for the survivors indicated that formulations of S-26741 alone and in formulations containing surfactants were cumulatively irritating. The gel formulations containing

S-26741 alone and those with surfactants as penetration enhancers were cumulatively irritating.

The sensitization potential of S-26741 gel formulations was explored using a complex series of experiments. In order to distinguish irritant from sensitization responses, initial studies were conducted to determine a non-irritating dose in the guinea pig. The non-irritating dose was used in the sensitization study; therefore, any dermal reaction could be identified as a sensitization response rather than a primary irritant response. The cumulative irritation/sensitization potential of the formulations was evident by the sixth induction dose. Dermal reactions (erythema and edema) were observed at the induction application site and S-26741-related clinical signs and death occurred in all groups treated with active formulations. Therefore, the induction phase was terminated after 6 doses (instead of 9) and the animals entered a 2-week period during which the mechanism for immunologic recognition could develop. At the end of this 2 week period, all animals were challenged by a single application of S-26741 formulations or S-26741/surfactant formulations to a naive dermal site (distal to the induction site). In general, a sensitization reponse has occurred if any animal has erythema or edema at the challenge site. In this study, 4 of 9 animals challenged with S-26741 had erythema and edema. The incidence of erythema and edema in groups challenged with S-26741/surfactant formulations was greater (eg, 5 of 7 or 8 of 9 animals). Thus, the data clearly demonstrates the sensitization potential of S-26741 formulations because of the relatively high incidence of positive responses to the challenge dose. The results of sensitization studies conducted in guinea pigs with HPMC gel formulations of S-26741 alone and with surfactant indicate that S-26741 is a sensitizer in guinea pigs. The addition of surfactant to the formulation appeared to potentiate the response since the incidence of sensitization was greater.

There were 33 safety assessment studies conducted in support of the S-26741 transdermal development program. In general, the transdermal formulations of S-26741 were cumulatively irritating in rabbits and contact sensitizers in guinea pigs. These dermal reactions probably resulted in enhanced and variable penetration of S-26741 because of disruption of the stratum corneum, the rate-limiting barrier for delivery of S-26741. Evidence for enhanced dermal penetration of S-26741 included clinical signs of S-26741 intoxication (eg, diarrhea, tremors) and death. The

incidence/severity of toxicity generally correlated with severity of the dermal reactions. Additional evidence for enhanced transdermal delivery is provided by review of the data for S-26741 patch residual. The variable dermal delivery of S-26741 would constitute a major obstacle to the conduct of a valid subchronic IND toxicology study. That is, differentiation of dose groups would be difficult and it is likely that most animals would eventually develop dermal irritation/sensitization and probably die from S-26741 intoxication. Therefore, this study would not provide the basis to select formulations for human clinical trials.

Table 2

Primary Skin Irritation Studies Conducted For S-26741 Project

RIKER Exp. No.	FORMULATION			FINDINGS
	S-26741	TYPE	ENHANCER	
0386EB0576	0%	Solution	0.35% Docusate Na	Non-irritating (Score 0.0/8.0)
0386EB0577	0%	Solution	0.33% Na Lauryl Sulfate	Non-irritating (Score 0.0/8.0)
0386EB0578	0%	Solution	1.0% K Laurate	Non-irritating (Score 0.0/8.0)
0386EB0633	50%	Microporous Membrane	0.33% Na Lauryl Sulfate	Slightly irritating (Score 0.9/8.0)
0386EB0634	50%	Microporous Membrane	0.35% Docusate Na	Minimally irritating (Score 0.3/8.0)
0386EB0635	50%	Gel	0.35% Docusate Na	Slightly irritating (Score 1.1/8.0)
0386EB0636	50%	Gel	0.33% Na Lauryl Sulfate	Slightly irritating (Score 0.8/8.0)
0386EB0637	50%	Solution	0.35% Docusate Na	Non-irritating (Score 0.0/8.0)
0386EB0638	50%	Solution	0.33% Na Lauryl Sulfate	Non-irritating (Score 0.0/8.0)
0386EB0669	50%	Solution	0.35% Docusate Na + 0.125% N-decyl Methyl Sulfoxide	Minimally irritating (Score 0.1/8.0)
0386EB0670	50%	Solution	0.5% Na Lauryl Sulfate	Minimally irritating (Score 0.1/8.0)
0386EB0671	50%	Solution	0.5% Na Myristyl Ether Sulfate	Minimally irritating (Score 0.2/8.0)
0386EB0672	50%	Solution	0.5% Na Octyl Sulfate	Minimally irritating (Score 0.2/8.0)

Table 3

S-26741

Guinea Pig Skin Sensitization Study Design

Group	Induction Phase (9 Topical Applications - Same Site)	Challenge Phase
I	Adjuvant/Drug only	Drug
II	Adjuvant/Drug + S.L.S.	Drug + S.L.S.
III	Adjuvant/Drug + S.D.	Drug + S.D.
IV	NO Adjuvant/Drug only	Drug
V	NO Adjuvant/Drug + S.L.S.	Drug + S.L.S.
VI	NO Adjuvant/Drug + S.D.	Drug + S.D.
VII	Adjuvant only	Drug
VIII	Adjuvant only	Drug + S.L.S.
IX	Adjuvant only	Drug + S.D.
X	Adjuvant/DNCB (Positive Control)	DNCB
XI	NO Adjuvant/DNCB (Positive Control)	DNCB

NOTE: Each group used 10 animals
S.L.S. - sodium lauryl sulfate
S.D. - sodium docusate
DNCB: 2,4-dinitrochlorobenzene
Adjuvant: Freund's Complete Adjuvant

Table 4

Summary of Sensitization Study Results

	# Animals Positive	Initial Dose Mean Score	Mean Score of Induction Doses	Mean Score of Challenge Doses
50% S-26741 - no adjuvant	4/9	0	0.03	0.22
50% S-26741 - with adjuvant	4/9	0	0.04	0.25
50% S-26741 - sham control	NA	NA	NA	0
30% S-26741 + 0.21% Docusate Sodium - no adjuvant	5/7	0	0.29	1.04
30% S-26741 + 0.21% Docusate Sodium - with adjuvant	7/8	0	0.30	1.06
30% S-26741 + 0.21% Docusate Sodium - sham control	NA	NA	NA	0
30% S-26741 + 0.198% Sodium Lauryl Sulfate - no adjuvant	8/9	0	0.38	1.17
30% S-26741 + 0.198% Sodium Lauryl Sulfate - with adjuvant	6/8	0	0.41	0.88
30% S-26741 + 0.198% Sodium Lauryl Sulfate - sham control	NA	NA	NA	0.05
DNCB positive control - no adjuvant	10/10	0	0.23	1.33
DNCB positive control - with adjuvant	10/10	0	0.38	1.70

DNCB: 2,4-dinitrochlorobenzene
NA: not applicable

APPENDIX A

LIST OF SAFETY EVALUATION STUDIES

Study Number	Title
0385RD0449	Minimum Toxic Dose Study with S-26741, Pyridostigmine Bromide in Beagle Dogs
0385AB0412	Acute Dermal Toxicity Study with S-26741, Lot 653035 (Pyridostigmine Bromide) in Albino Rabbits
0385AR0413	Acute Oral Toxicity Study with S-26741, Lot 65305 in Albino Rats
0385EB0414	Primary Skin Irritation with S-26741, Lot 653035 in Albino Rabbits
0386EB0576	Primary Skin Irritation Test with 0.35% Docusate Sodium (w/v solution in water and 5% glycerin) in Albino Rabbits
0386EB0577	Primary Skin Irritation Test with 0.33% Sodium Lauryl Sulfate (w/v solution in water and 5% glycerin) in Albino Rabbits

Study Number	Title
0386EB0578	Primary Skin Irritation Test with 1% Potassium Laurate (w/v solution in water and 5% glycerin) in Albino Rabbits
0386EB0633	Primary Skin Irritation Test with 50% S-26741 + 0.33% Sodium Lauryl Sulfate in a Microporous Membrane in Albino Rabbits
0386EB0634	Primary Skin Irritation Test with 50% S-26741 + 0.35% Docusate Sodium in a Microporous Membrane in Albino Rabbits
0386EB0635	Primary Skin Irritation Test with 50% S-26741 + 0.35% Docusate Sodium in a Gel in Albino Rabbits
0386EB0636	Primary Skin Irritation Test with 50% S-26741 + 0.33% Sodium Lauryl Sulfate in a Gel in Albino Rabbits
0386EB0637	Primary Skin Irritation Test with 50% S-26741 + 0.35% Docusate Sodium in Solution with Water and 5% Glycerin in Albino Rabbits
0386EB0638	Primary Skin Irritation Test with 50% S-26741 + 0.33% Sodium Lauryl Sulfate in Solution with Water and 5% Glycerin in Albino Rabbits
0386EB0669	Primary Skin Irritation Test with 50% S-26741 + 0.35% Docusate Sodium + 0.125% N-decyl Methyl Sulfoxide in Solution with Water and 5% Glycerin in Albino Rabbits

Study Number	Title
03686EB067	Primary Skin Irritation Test with 50% S-26741 + 0.5% Sodium Lauryl Sulfate in Solution with Water and 5% Glycerin in Albino Rabbits
0386EB0671	Primary Skin Irritation Test with 50% S-26741 + 0.5% Sodium Myristyl Ether Sulfate in Solution with Water and 5% Glycerin in Albino Rabbits
0386EB0672	Primary Skin Irritation Test with 50% S-26741 + 0.5% Sodium Octyl Sulfate in Solution with Water and 5% Glycerin in Albino Rabbits
0387EG0053	Repeat Skin Irritation Study with Hydroxypropylmethylcellulose Gel Containing 50% Pyridostigmine Bromide (Lot 4588) in Albino Guinea Pigs
0387EG0056	Repeat Skin Irritation Study with Hydroxypropylmethylcellulose Gel Containing 30% Pyridostigmine Bromide and 0.21% Docusate Sodium (Lot 4589) in Albino Guinea Pigs
0387EG0059	Repeat Skin Irritation Study with Hydroxypropylmethylcellulose Gel Containing 30% Pyridostigmine Bromide and 0.198% Sodium Lauryl Sulfate (Lot 459) in Albino Guinea Pigs
0387EB0073	Repeat Skin Irritation Test with Hydroxypropylmethylcellulose Gel Containing 50% Pyridostigmine Bromide (Lot FN4588) in Albino Rabbits

Study Number	Title
0387WB0074	Repeat Skin Irritation Test with Hydroxypropylmethylcellulose Gel Containing 30% Pyridostigmine Bromide and 0.21% Docusate Sodium (Lot FN4589) in Albino Rabbits
0387EB0075	Repeat Skin Irritation Test with Hydroxypropylmethylcellulose Gel Containing 30% Pyridostigmine Bromide and 0.198% Sodium Lauryl Sulfate (Lot FN4590) in Albino Rabbits
0385MG0411	Sensitization Study with S-26741 (Lot 653035) in Guinea Pigs
0386MS0737	Sensitization Study with 50% S-26741 + 0.33% Sodium Lauryl Sulfate in a Microporous Membrane in Yorkshire Swine
0386MG0769	Sensitization Study with 50% S-26741 + 0.33% Sodium Lauryl Sulfate in Solution with Water and 5% Glycerin in Albino Guinea Pigs
0387MG0051	Sensitization Study with Hydroxypropylmethylcellulose Gel Containing 50% Pyridostigmine Bromide in Albino Guinea Pigs
0387MG0052	Sensitization Study with Hydroxypropylmethylcellulose Gel Containing 50% Pyridostigmine Bromide (Lot FN4588) in Albino Guinea Pigs

Study Number	Title
0387MG0054	Sensitization Study with Hydroxypropylmethylcellulose Gel Containing 30% Pyridostigmine Bromide and 0.21% Docusate Sodium in Albino Guinea Pigs
0387MG0055	Sensitization Study with Hydroxypropylmethylcellulose Gel Containing 30% Pyridostigmine Bromide and 0.21% Docusate Sodium in Albino Guinea Pigs
0387MG0057	Sensitization Study with Hydroxypropylmethylcellulose Gel Containing 30% Pyridostigmine Bromide and 0.198% Sodium Lauryl Sulfate in Albino Guinea Pigs
0387MG0058	Sensitization Study with Hydroxypropylmethylcellulose Gel Containing 30% Pyridostigmine Bromide and 0.198% Sodium Lauryl Sulfate in Albino Guinea Pigs
1187MK0018	Cytotoxicity Test - Agar Overlay with Microporous Membranes Using L-929 Mouse Fibroblasts

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1. Schalbrueter, K.U., Schultz, K.H., and Wood, J.M. Induction of contact dermatitis in guinea pigs by quaternary ammonium compounds: the mechanism of antigen formation. Environ. Health Persp. 70:229-238, 1986.

Minimum Toxic Dose Study

with S-26741, Pyridostigmine Bromide (Lot 653035)

in Beagle Dogs

Experiment No: 0385RD0449

Conducted At: Pathology and Toxicology
Riker Laboratories, Inc.
St. Paul, Minnesota

Dates Conducted: October 14, 1985 to November 12, 1985

Conducted By:

Gene L. Harris 2/6/86

G. L. Harris, BS Date
Advanced Toxicologist
Study Director

Reviewed By:

Karen D O'Malley 2/6/86

K. D. O'Malley, BS Date
Senior Toxicologist
Acute Toxicology

K L Ebbens 2/6/86

K. L. Ebbens, BS Date
Supervisor, Toxicology Testing

dc: R. T. Catherall
M. W. Downing
K. L. Ebbens
A. K. Mitra
M. J. Westfall (2)
Path/Tox Files

Summary

The acute oral minimum toxic dose study with S-26741, Pyridostigmine Bromide, Lot 653035, was conducted from October 14, 1985 to November 12, 1985 at Riker Laboratories, Inc., St. Paul, Minnesota using male and female Beagle dogs ranging in body weight from 14.8 to 19.8 kilograms. The test article was administered orally by gelatin capsule at dose levels of 90, 30, 10 and 1 mg/kg body weight with mortalities of 1/1, 0/1, 0/1 and 0/1 noted respectively.

The pharmacotoxic signs noted during the 14 day study were salivation, emesis, labored respiration, lacrimation, ataxia, diarrhea and involuntary muscle twitching.

The onset of pharmacotoxic signs occurred between 30 minutes and 2 hours after dosing and recovery was complete in all surviving animals by day one. The one mortality noted occurred at two hours after dosing. Body weights of all study animals essentially stayed the same during the study within $\pm$ 0.1 kilogram. The animal at the 1 mg/kg dose level appeared normal throughout the entire 14 day observation period and the 1 mg/kg level is the only level in which no adverse reactions were noted. Therefore, based on the results of this test a minimum toxic dose would be between 1 and 10 mg/kg and 1 mg/kg would be considered a no effect level when S-26741 is administered orally by gelatin capsule to Beagle dogs.

Introduction

The objective of this study was to determine a minimum toxic dose of S-26741 in dogs, so that dose levels may be chosen, for a dog rangefinding toxicity study with this drug to follow. This study was conducted in accordance with the Food and Drug Administration's Good Laboratory Practice Regulation of 1978. The raw data generated by the Study Director and the final report will be stored in the conducting laboratory's archives.

Method and Results

Beagle dogs[a], approximately two years old were used for this test. All animals were held under quarantine prior to testing and only animals which appeared to be in good health and suitable as test animals at the initiation of the study were used. Each dog was individually housed in a 3' x 8' run with cement floors in a temperature, humidity and light controlled room. The dogs were permitted a standard laboratory diet[b] plus water ad libitum throughout the study.

The dogs (one per dose level) were administered the test material at preselected dosage levels. All doses were administered by single administration orally by gelatin capsule[c] (size 13).

After oral administration of the test article the dogs were observed for any adverse reactions and mortality for the following 14 days. Initial, seven day and final body weights, mortalities and adverse reactions were recorded and are listed in Table 1. The protocol, principal personnel involved in the study, composition characteristics and Quality Assurance statement are contained in Appendices I - IV.

[a] Laboratory Research Enterprises, Kalamazoo, MI
[b] Wayne's Dog Food, Continental Grain, Chicago, IL
[c] Manufactured by Torpac Limited, Lot #3043

Table 1

Acute Oral Minimum Toxic Dose Study - Beagle Dogs

with S-26741, Pyridostigmine Bromide

Dose (mg/kg)	Animal No.	Sex	Body Weight (kg) Test Day Number 0	7	14	Number Dead Number Tested	Percent Dead
90	4D124	F	15.6	(2-4 hours)	-	1/1	100
30	4D114	M	19.8	19.9	19.9	0/1	0
10	4D122	F	14.8	14.9	14.9	0/1	0
1	4D122*	F	14.9	14.9	14.8	0/1	0

Note: Figure in parenthesis indicates time of death.

* Animal received 1 mg/kg dose following a 14 day recovery period from the original 10 mg/kg dose.

Summary of Adverse Reactions

Dose mg/kg	Sex	Reaction	Observation Periods Number Affected/Number Dosed Minutes 1-30	60	120	Day 1	2 - 14
90	F	Salivation	1/1	1/1	1/1	0/0	-
		Emesis*	1/1	0/1	1/1	0/0	-
		Ataxia	1/1	1/1	1/1	0/0	-
		Labored Respiration	1/1	1/1	1/1	0/0	-
		Lacrimation	1/1	1/1	1/1	0/0	-
		Diarrhea		1/1	0/1	-	-
		Involuntary muscle twitching			1/1	0/0	-
		Prostration			1/1	0/0	-
30	M	Salivation	1/1	1/1	1/1	0/1	-
		Emesis*		1/1	1/1	0/1	-
		Diarrhea			1/1	0/1	-
10	F	Ataxia			1/1	0/1	-
		Emesis*			1/1	0/1	-
		Lacrimation			1/1	0/1	-
1	F	No adverse reactions were noted throughout the 14 day study at this level.					

* = Emesis noted was clear and foamy.
- = No observed untoward reactions

Protocol for Riker Study No. 0385RD0449

TITLE: Protocol for a Minimal Toxic Dose (MTD) Study with S-26741, Pyridostigmine Bromide (Lot 6503035) when given orally in dogs.

OBJECTIVE: To determine levels for a Dose Rangefinder Study.

TEST ARTICLE: S-26741, Pyridostigmine Bromide, Lot 6503035

SPONSOR: Riker Laboratories, Inc., 3M Company, St. Paul, Minnesota, 55144

TESTING FACILITY: Pathology and Toxicology Department, Riker Laboratories, Inc., St. Paul, Minnesota, 55144

TEST INTERVAL: October, 1985* through January, 1986.

TEST SYSTEM: Beagle dogs approximately 2 years of age on dose day one. The animals will be housed in 3 ft x 8 ft runs with cement floors. The room is temperature and humidity controlled with the lights on a 12 hour light/dark cycle. Each animal will be given a single dosage only.

JUSTIFICATION FOR SELECTION OF THE TEST SYSTEM: Beagle Dogs will be used because the rangefinder study will be done in this strain of dogs.

TEST SYSTEM IDENTIFICATION: Each animal will be assigned a number which will be indicated on the outside cage and vendor ID number tatooed inside the ear.

RANDOMIZATION OF TEST SYSTEM: The dogs will be randomized to treatment and dose groups at the discretion of the Study Director.

DIET AND OTHER COMMERCIALLY AVAILABLE FORMULATION/ANALYTICAL SPECIFICATIONS: Water and Wayne's Dog Food will be available ad libitum throughout the study.

Chemical analysis of the test article S-26741 will be determined prior to dosing. Analytical report will be on file in the raw data.

*NOTE: The test interval are the proposed dates from study initiation to the issuance of the report.

continued .....

574

Study No. 0385RD0449
page -2-

DOSAGE LEVELS, ROUTE, GROUP SIZE ETC.: The test article will be given a single administration orally by gelatin capsule to one dog per dose level as indicated below:

Dose Level	Group Size
30 mg/kg	1 dog of either sex
10 mg/kg	1 dog of either sex

Any additional dosage levels, or changes in dosage levels, if needed, will be at the discretion of the Study Director.

CLINICAL OBSERVATIONS: The animals will be observed frequently during the day of compound administration twice daily thereafter throughout the 14 day test interval for evidence of treatment related toxicity. Body weights will be recorded initially and at the end of the 14 day observation period.

TISSUE PATHOLOGY: No gross necropsies will be performed on these animals. This will be addressed in the rangefinder study.

FINAL REPORT: Mortality between groups will be compared as well as a comparison of treatment related toxicities. The proposed date for the final report is 1-3 months after the observation period. All raw data generated by the Study Director and the final report will be stored in the Riker Laboratories Archives, St. Paul, Minnesota.

RE CH 10/8/85

K. Gene Harris 10/8/85
Study Director Date

Supervisor, Toxicology Testing Date 10/8/85

Riker Experiment No. Q385RD0449

575

Appendix I (concluded)
Deviations and/or Amendments to Protocol

1. The lot number for the test material S-26741 is 653035. There is a typo in the original protocol.

G. L. Harris — Study Director — 10/14/85 — Date

2. Body weights will also be taken on day seven. Rational for change: weekly body weights are generally S.O.P. for studies of this nature.

G. L. Harris — Study Director — 10/21/85 — Date

3. An additional dose level of 1 mg/kg will be dosed to dog #4D122 (the original 10 mg/kg dog) after the first 14-day study period is terminated. This is being done to try and find a no effect level for the compound.

G. L. Harris — Study Director — 10/28/85 — Date

4. Completion date is extended to 2/86 due to the addition of a 1 mg/kg dose level.

G. L. Harris — Study Director — 2/6/86 — Date

5. _____

Study Director — Date

APPENDIX II

Principal Participating Personnel Involved in the Study

Name	Function
G. L. Harris, BS	Advanced Toxicologist Study Director
G. E. Hart	Sr. Laboratory Technician Acute Toxicology
K. D. O'Malley, BS	Senior Toxicologist Acute Toxicology
K. L. Ebbens, BS	Supervisor Toxicology Testing
G. C. Pecore	Supervisor Animal Laboratory
L. O. Wiseth	Sr. Laboratory Technician Acute Toxicology

APPENDIX III - A

Test and/or Control Article Characterization

for

S-26741 (PYRIDOSTIGMINE BROMIDE), Lot# 653035

1. The identity strength, uniformity, composition, purity or other pertinent characterizations of the test and/or control substances have been determined and documented as of 8/19/85.

2. The method of synthesis or origin of the test and control substances, including their amount and the method of bioassay (if applicable) is documented.
yes X no ___ (~~NOT APPLICABLE~~) RE GH 1/30/86.

3. The stability of the test and/or control substances have been determined or will be determined as of ___.
Raw material stability (NOT REQUIRED FOR ACUTE STUDIES) - See Pathology/T SOP's

The above information and documentation are located in the sponsor's records.

Arnold G. Roth 1/30/86
Sponsor Date

578

3M

Riker St. Paul Drug Clearance Certificate

☒ Original Clearance ☐ Re-Clearance

Purpose: Reference Standard		
Sample Description: Pyridostigmine Bromide		
Compound/Lot No: Hoffman-LaRoche Lot #653035	Batch Size: 200 gm	RFA: 11055
Reference Standard Lot: -	Previous References: -	

Test Results ☒ Full Clearance ☐ Selected Tests

Assay: 99.19% (on the dried basis)

Loss on Drying: 0.51%

Identification:

Infrared Spectrum: Spectrum IR 1492 agrees with USP Reference Standard Spectrum IR 1491.

Ultraviolet Spectrum: Spectrum UV 1565 agrees with USP Reference Standard Spectrum UV 1564.
Respective absorptivity 103.0% of USP Reference Standard.

Identification C: Responds to identification test.

Identification D: Responds to test for Bromide.

Melting Range: 154.2° - 155.0°

Residue on Ignition: 0

Note. Specifications or reference value in parenthesis

* Not formal clearance specification

Comments

Reference USP XXI

Analytical Review	Date	Quality Control Approval	Date
C. A. Kolars	10-14-85		9 JAN 86

Form 22162 Rev.

APPENDIX IV

QUALITY ASSURANCE STATEMENT

Acute Toxicology Laboratory Studies

Study No.: 0385R00449

This short term study was audited by Compliance Audit and the final report examined against the raw data on February 7, 1986. The results of the audit were reported to the study director and to management on February 7, 1986.

In addition to the data audit, different significant phases for studies underway in the Acute Toxicology Laboratory are inspected weekly on a recurring cyle, and the facilities are examined by Compliance Audit on a three month schedule.

D.M. Markowitz
Compliance Audit

2-7-86
Date

Acute Dermal Toxicity Study

with S-26741, Lot 653035 (Pyridostigmine Bromide)

in Albino Rabbits

Experiment No: 0385AB0412

Conducted At: Pathology and Toxicology
Riker Laboratories, Inc.
St. Paul, Minnesota

Dates Conducted: October 15, 1985 to January 28, 1986

Conducted By: Glen L. Harris 2/6/86
G. L. Harris, BS Date
Advanced Toxicologist
Study Director

Reviewed By: Karen D O'Malley 2/6/86
K. D. O'Malley, BS Date
Senior Toxicologist
Acute Toxicology

K. L. Ebbens 2/7/86
K. L. Ebbens, BS Date
Supervisor, Toxicology Testing

dc: R. T. Catherall
M. W. Downing
K. L. Ebbens
A. K. Mitra
M. J. Westfall (2)
Path/Tox Files

Summary

The acute dermal toxicity study with S-26741, Pyridostigmine Bromide (Lot 653035), was conducted at Riker Laboratories, Inc., St. Paul, Minnesota from October 15, 1985 to January 28, 1986 using five male and five female rabbits, per dose level, ranging in body weight from 1.6 to 2.1 kilograms. The test article was administered undiluted by dermal application at dosage levels of 2,000, 1,000, 500, 250, 80 and 63 mg/kg body weight and mortalities of 10/10, 10/10, 10/10, 10/10, 5/10, and 2/10 respectively occurred within 24 hours of dosing. The pharmacotoxic signs noted were salivation, tremors, involuntary muscle twitching, clonic convulsions, hypoactivity, ataxia, prostration and diarrhea. All surviving animals appeared normal by the observation on day one and remained normal for the duration of the 14 day study. No skin irritation was noted in the surviving rabbits and all surviving rabbits gained weight during the study. A gross necropsy was performed on all animals and the results revealed no visible lesions with the exception of one male rabbit at the 500 mg/kg dose level which had blood around the anus at necropsy. Based on the results of this test the dermal LD50 of S-26741 is 80 mg/kg when applied undiluted to male and female albino rabbits.

Introduction

The objective of this study was to determine the acute dermal LD50 of S-26741, Pyridostigmine Bromide (Lot 653035), in male and female albino rabbits. This study was conducted in accordance with the Food and Drug Administration's Good Laboratory Practice Regulation of 1978. The raw data generated by the Study Director and the final report are stored in the conducting laboratory's archives.

Method and Results

Young albino rabbits[a] were used in this test. All animals were held under quarantine for several days prior to testing with only animals which appeared to be in good health and suitable as test animals at the initiation of the study used. The rabbits were housed in suspended, wire-mesh cages in temperature and humidity controlled rooms and permitted a standard laboratory diet[b] plus water ad libitum.

The trunk of five male and five female rabbits, per dose level, was clipped free of hair with an electric clippper and the skin of each animal was then abraded. The test article was applied on the surface of the skin for a one day exposure period at dosage levels of 2,000, 1000, 500, 250, 80 and 63 mg/kg. The trunk of each animal was wrapped with impervious plastic sheeting[c] to occlude the test article and a flexible plastic collar was fitted on the rabbits to prevent test article ingestion. After the one day exposure period the plastic wrapping and collar were removed from each animal and all residual test article washed off with water. The animals were then returned to their cages and observed daily for untoward behavioral reactions and skin reactions for the following 14 days. Initial day seven and final body weights, mortalities (Table 1) and adverse reactions (Table 2) were recorded. A necropsy was conducted on all animals that died during the study as well as those euthanatized at the end of the 14 day observation period (Table 1). The protocol, principal personnel involved in the study, composition characteristics and Quality Assurance statement are contained in Appendices I - IV.

[a] Hazleton-Dutchland, Inc., Denver, PA
[b] Purina Rabbit Chow, Ralston Purina, St. Louis, MO
[c] 10 x 22 x .02 extra clear polyethylene sleeves, PPC Industries, Inc., Wheeling, IL

TABLE 1

ACUTE DERMAL TOXICITY STUDY - ALBINO RABBITS

with S-26741, Lot 653035

Mortality, Necropsy and Body Weight Data

Dose[a] (mg/kg)	Sex	Animal Number	Individual Body Weights (kg) Test Day Number: 0	7	14	Number Dead / Number Tested	Percent Dead
2,000	M	5B1650	1.8	(1 hour)	-	5/5	100
		5B1652	1.9	(1 hour)	-		
		5B1655	1.6	(1 hour)	-		
		5B1651	1.9	(1 hour)	-		
		5B1653	1.7	(1 hour)	-		
2,000	F	5B1639	1.6	(1 hour)	-	5/5	100
		5B1640	1.7	(1 hour)	-		
		5B1641	1.7	(1 hour)	-		
		5B1627	1.9	(1 hour)	-		
		5B1630	1.6	(1 hour)	-		
1,000	M	5B1826	1.8	(1 hour)	-	5/5	100
		5B1829	1.9	(1 hour)	-		
		5B1832	1.8	(1 hour)	-		
		5B1827	1.7	(2 hours)	-		
		5B1830	1.8	(2 hours)	-		
1,000	F	5B1805	2.0	(2 hours)	-	5/5	100
		5B1803	1.7	(2 hours)	-		
		5B1800	1.8	(1 hour)	-		
		5B1804	2.0	(1 hour)	-		
		5B1807	1.8	(1 hour)	-		

TABLE 1 (continued)

ACUTE DERMAL TOXICITY STUDY - ALBINO RABBITS

with S-26741, Lot 653035

Mortality, Necropsy and Body Weight Data

Dose[a] (mg/kg)	Sex	Animal Number	Individual Body Weights (kg) Test Day Number: 0	7	14	Number Dead / Number Tested	Percent Dead
500	M	5B1833	2.0	(2 hours)	-	5/5	100
		5B1828	1.8	(2 hours)	-		
		5B1831	1.9	(3 hours)	-		
		5B1834*	1.8	(2 hours)	-		
		5B1814	1.8	(1 hour)	-		
500	F	5B1810	1.9	(3 hours)	-	5/5	100
		5B1763	1.9	(2 hours)	-		
		5B1764	1.8	(1 hour)	-		
		5B1765	1.7	(1 hour)	-		
		5B1766	1.7	(1 hour)	-		
250	M	5B1817	2.0	(2 hours)	-	5/5	100
		5B1820	2.0	(2 hours)	-		
		5B1823	1.9	(2 hours)	-		
		5B1815	1.9	(2 hours)	-		
		5B1818	2.0	(2 hours)	-		
250	F	5B1769	1.9	(2 hours)	-	5/5	100
		5B1772	2.0	(2 hours)	-		
		5B1767	1.8	(2 hours)	-		
		5B1770	1.9	(2 hours)	-		
		5B1773	2.0	(1 hour)	-		

TABLE 1 (concluded)

ACUTE DERMAL TOXICITY STUDY - ALBINO RABBITS

with S-26741, Lot 653035

Mortality, Necropsy and Body Weight Data

Dose[a] (mg/kg)	Sex	Animal Number	Individual Body Weights (kg) Test Day Number: 0	7	14	Number Dead / Number Tested	Percent Dead
80	M	6B0085	1.7	(2 hours)	-	4/5	80
		6B0080	1.8	2.0	2.2		
		6B0083	1.7	(4 hours)	-		
		6B0086	2.1	(2 hours)	-		
		6B0082	1.8	(Day 1)	-		
80	F	6B0027	1.8	2.0	2.2	1/5	10
		6B0003	1.8	2.1	2.4		
		6B0008	1.9	(1-30 min)	-		
		6B0012	1.9	2.2	2.4		
		6B0035	1.8	2.0	2.2		
63	M	5B1821	2.0	(1 hour)	-	2/5	40
		5B1824	2.1	2.1	2.6		
		5B1816	2.0	2.1	2.5		
		5B1819	1.8	1.9	2.3		
		5B1822	1.9	(2-4 hours)	-		
63	F	5B1768	2.0	2.2	2.5	0/5	0
		5B1771	1.9	2.0	2.3		
		5B1774	1.9	2.0	2.5		
		5B1739	1.9	2.0	2.4		
		5B1742	1.8	2.0	2.2		

[a] The test article was administered undiluted.

The acute dermal LD50 is 80 mg/kg in male and female albino rabbits.

Necropsy

Necropsies were performed on all animals at the end of the 14 day study and no visible lesions were noted with the exception of one male rabbit at the 500 mg/kg dose level (marked with an asterisk). which had blood around the anus at necropsy.

Note: The time in parenthesis indicates the time of death.

Table 2

ACUTE DERMAL TOXICITY STUDY - ALBINO RABBITS
with S-26741, Lot 653035

Summary of Reactions

Dose mg/kg	Sex	Reactions	Observation Periods: Minutes 1-30	Minutes 60	Minutes 120-240	Days 1	2	3	4	5	6	7	8	9	10	11	12	13	14
2,000	M																		
		Salivation		5/5	*														
2,000	F																		
		Salivation		5/5	*														
1,000	M																		
		Salivation		5/5															
		Clonic convulsions		5/5	*														
		Tremors		1/5															
1,000	F																		
		Salivation		5/5															
		Clonic convulsions		5/5	*														
		Tremors		3/5															
500	M																		
		Salivation		5/5	2/2														
		Clonic convulsions		5/5	2/2	*													
		Tremors			2/2														
		Prostration		1/5	2/2														
500	F																		
		Salivation		5/5	0/1														
		Clonic convulsions		4/5	0/1														
		Tremors		4/5	0/1	*													
		Prostration			1/1														
		Ataxia		1/5	0/1														

Table 2 (concluded)

ACUTE DERMAL TOXICITY STUDY - ALBINO RABBITS
with S-26741, Lot 653035

Summary of Reactions

Dose mg/kg	Sex	Reactions	Observation Periods Number Affected/Number Dosed													
			Minutes													
			1-30	60	120-240	1	2	3	4	5	6	7	8	9	10	11	12	13	14
250	M																		
		Salivation.		5/5															
		Clonic convulsions		5/5	*														
		Tremors		5/5															
250	F																		
		Salivation		5/5	1/1														
		Clonic convulsions		4/5	1/1	*													
		Tremors		4/5	1/1														
80	M																		
		Salivation	4/5	4/5	2/3	0/1													
		Involuntary muscle twitching	3/5	4/5	2/3	0/1													
		Prostraton	2/5	3/5	2/3	0/1													
		Diarrhea			2/3	0/1													
80	F																		
		Salivation	1/5	0/4															
63	M																		
		Salivation		3/5	2/4	0/3													
		Tremors		2/5	3/4	0/3													
		Prostration		2/5	1/4	0/3													
		Hypoactivity			2/4	0/3													
63	F																		
		Salivation			1/5	0/5													

* = Total death

Riker Experiment No.: 0385AB0412

APPENDIX I
PROTOCOL

588

TEST: Acute Dermal Toxicity Study

SPONSOR: 3M RIKER Division

CONDUCTED BY: **Safety Evaluation Laboratory, Riker Laboratories, Inc., St. Paul, Minnesota** RAT 64

TEST ARTICLE: S-26741, PYRIDOSTIGMINE BROMIDE, 653035

CONTROL ARTICLE:

PROPOSED STARTING/COMPLETION DATE OF TEST: 9/85 - 12/85

TEST SYSTEM: New Zealand White Albino Rabits: Sex ♂ & ♀ Number 5/sex

SOURCE: HAZELTON DUTCHLAND, DENVER, PA. Weight Range 1.5 - 3.0 Kg

OBJECTIVE: The objective of this study will be to characterize the acute dermal toxicity of the test article in albino rabbits. Rabbits were selected as the test system for their sensitivity of response, historical data, ease of handling and general availability.

METHOD: The animals will be housed in standard wire-mesh cages in temperature and humidity controlled rooms with food[a] and water offered *ad libitum*. Each animal will be assigned a numbered ear tag, which will correspond to a card affixed to the outside of the cage. The trunk of each animal will be clipped free of hair and the test article placed on the surface of the skin for a 24 hour exposure period as a single dosage of * mg/kg, however, if this dosage level does not adequately characterize the toxicity of the test article, additional animals will be administered the test article at supplemental dosage levels. Any additional dosage levels will be documented and filed with this protocol. The test article will be administered to the animals in the form received from the sponsor. The trunk of each animal will be wrapped with impervious plastic sheeting which will occlude the test article during the test period and a flexible plastic collar will be fitted on the animals to prevent test article ingestion. After administration of the test article, the animals will be returned to their cages for a 24 hour exposure period, after which time the plastic wrapping and test material will be removed from the dermal surface of the animals. The animals will be observed for untoward behavioral reactions during the exposure period and after removal of the test article, for the following 14 days with all observations recorded and maintained. General skin reactions will be noted after removal of the test article and periodically throughout the duration of the study. Initial and final body weights will also be recorded. A gross necropsy, which will include, but not be limited to, heart, lungs, liver, kidneys and general gastro-intestinal tract and will be conducted on all animals which die during the conduct of the study, as well as the animals which survive the study. Any gross abnormalities which are observed during the conduct of the necropsy will be recorded with specific mention to the organ and/or site observed. The acute median lethal dose (LD_{50}) of the test article will be calculated, if possible, using a probit analysis method at the end of the observation period. All raw data generated by the study director and the final report will be stored in the Riker Laboratories' Archive, St. Paul, Minnesota.

[a] Purina Rabbit Chow, Ralston Purina, St. Louis, Missouri

* = TO BE DETERMINED.

RECEIVED
SEP 18 1985
PATHOLOGY AND TOXICOLOGY

K L Eckdahl 9/16/85
Sponsor Date

Gene Hans 9/18/85
Study Director Date

Form 19171-2-B-PWO

Riker Experiment No. 0385AB0412

Appendix I (concluded)
Deviations and/or Amendments to Protocol

589

1. An initial dose level of 2,000 mg/kg will be administered. This dose level was chosen because no deaths or dermal irritation was noted in the primary skin irritation (0385EB0414) conducted previously.

G. L. Harris — Study Director — 10/14/85 — Date

2. Because of the mortalities noted at the 2,000 mg/kg dose level, dose levels of 1,000 and 500 mg/kg dose levels will be added to the study.

G. L. Harris — Study Director — 11/12/85 — Date

3. Because of mortalities noted at 500 mg/kg dose,levels will be added to this study until a LD50 is established.

G. L. Harris — Study Director — 11/14/85 — Date

4. Due to the additional dose levels that had to be added to this study the completion date of the test has been extended to 2/86.

G. L. Harris — Study Director — 2/3/86 — Date

5. _____

Study Director — Date

APPENDIX II

Principal Participating Personnel Involved in the Study

Name	Function
G. L. Harris, BS	Advanced Toxicologist Study Director
G. E. Hart	Sr. Laboratory Technician Acute Toxicology
K. D. O'Malley, BS	Senior Toxicologist Acute Toxicology
K. L. Ebbens, BS	Supervisor Toxicology Testing
G. C. Pecore	Supervisor Animal Laboratory
L. O. Wiseth	Sr. Laboratory Technician Acute Toxicology

APPENDIX III - A 591

Test and/or Control Article Characterization

for

S-26741, (PYRIDOSTIGMINE BROMIDE), Lot# 65305

1. The identity strength, uniformity, composition, purity or other pertinent characterizations of the test and/or control substances have been determined and documented as of 8/19/85.

2. The method of synthesis or origin of the test and control substances, including their amount and the method of bioassay (if applicable) is documented.

yes X no ___ ~~(NOT APPLICABLE)~~ RE EH 1/30/86

3. The stability of the test and/or control substances have been determined or will be determined as of ___.

Raw material stability (NOT REQUIRED FOR ACUTE STUDIES) - SEE PATHOLOGY SOP

The above information and documentation are located in the sponsor's records.

Sponsor 1/30/86 Date

Form 19793 PWO

592

Riker St. Paul Drug Clearance Certificate

3M

☒ Original Clearance ☐ Re-Clearance

Purpose: Reference Standard		
Sample Description: Pyridostigmine Bromide		
Compound/Lot No: Hoffman-LaRoche Lot #653035	Batch Size: 200 gm	RFA: 11055
Reference Standard Lot: -	Previous References: -	

Test Results ☒ Full Clearance ☐ Selected Tests

Assay: 99.19% (on the dried basis)

Loss on Drying: 0.51%

Identification:

Infrared Spectrum: Spectrum IR 1492 agrees with USP Reference Standard Spectrum IR 1491.

Ultraviolet Spectrum: Spectrum UV 1565 agrees with USP Reference Standard Spectrum UV 1564.
Respective absorptivity 103.0% of USP Reference Standard.

Identification C: Responds to identification test.

Identification D: Responds to test for Bromide.

Melting Range: 154.2° - 155.0°

Residue on Ignition: 0

Note Specifications or reference value in parenthesis

* Not formal clearance specification

Comments

Reference USP XXI

Analytical Review	Date	Quality Control Approval	Date
C. A. Kolars	10-14-85		9 JAN 8

APPENDIX IV

QUALITY ASSURANCE STATEMENT

Acute Toxicology Laboratory Studies

Study No.: 0385ABD412

This short term study was audited by Compliance Audit and the final report examined against the raw data on FEBRUARY 7, 1986. The results of the audit were reported to the study director and to management on FEBRUARY 7, 1986.

In addition to the data audit, different significant phases for studies underway in the Acute Toxicology Laboratory are inspected weekly on a recurring cyle, and the facilities are examined by Compliance Audit on a three month schedule.

D. M. Marbaugh

Compliance Audit

2-7-86

Date

Acute Oral Toxicity Study with S-26741, Lot 653035 in Albino Rats

Experiment No: 0385AR0413

Conducted At: Pathology and Toxicology
Riker Laboratories, Inc.
St. Paul, Minnesota

Dates Conducted: September 24, 1985 to November 19, 1985

Conducted By:

Jane L. Harris 12/30/85
G. L. Harris, BS Date
Advanced Toxicologist
Study Director

Karen D O'Malley 1/16/86
K. D. O'Malley, BS Date
Senior Toxicologist
Acute Toxicology

Reviewed By:

K Ebbens 1/17/86
K. L. Ebbens, BS Date
Supervisor, Toxicology Testing

dc: R. T. Catherall
M. W. Downing
K. L. Ebbens
A. K. Mitra
M. J. Westfall (2)
Path/Tox Files

Summary

The acute oral toxicity study with S-26741, Lot 653035 (Pyridostigmine Bromide) was conducted from September 24, 1985 to November 19, 1985 at Riker Laboratories, Inc., St. Paul, Minnesota using male and female albino rats ranging in body weight from 200-385 grams. The test article was administered by gastric intubation at dose levels of 80, 60, and 40 mg/kg body weight with mortalities of 10/10, 6/10, and 2/10 respectively. The untoward behavioral reactions noted during the 14 day observation period generally consisted of hypoactivity, prostration, ataxia, clonic convulsions, salivation, involuntary muscle twitching, tremors, and red lacrimation. The onset of pharmacotoxic signs and death was rapid as they occurred in most rats within one hour of dosing. Generally, recovery was complete in all surviving animals by day three. Body weight gains were noted in all animals that survived the study with the exception of one rat with malocclusion. Necropsies performed on all animals on study revealed three rats with hyperemic lungs and one rat with hemorrhage of the small intestine, all of which were in the 60 mg/kg dose group. All other rats had no visible lesions. The acute oral LD50 of S-26741, Lot 653035, is 52 mg/kg with 95% confidence limts of 42-61 mg/kg.

Introduction

The objective of this study was to determine the acute oral LD50 of S-26741, Lot 653035 (Pyridostigmine Bromide), in albino rats. This study was conducted in accordance with the Food and Drug Administration's Good Laboratory Practice Regulation of 1978. The raw data generated by the Study Director and the final report are stored in the conducting laboratory's archives.

Method and Results

Young albino rats[a] were used in this test. All animals were held under quarantine for several days prior to testing with only animals which appeared to be in good health and suitable as test animals at the initiation of the study used. The rats were housed in suspended, wire-mesh cages in temperature and humidity controlled rooms and permitted a standard laboratory diet[b] plus water ad libitum except during the 16 hour period immediately prior to gastric intubation when food was withheld.

The rats were administered the test material as a solution in sterile distilled water at preselected dosage levels. All doses were administered at a constant volume of 10 ml/kg directly into the stomachs of the rats using a hypodermic syringe equipped with a ball-tipped intubating needle[c]. The dosage levels administered were 80, 60, and 40 mg/kg.

After gastric administration of the test article, the rats were returned to their cages and observed for the following 14 days. Initial, seven day and final body weights, mortalities (Table 1) and adverse reactions (Table 2) were recorded. A necropsy was conducted on all animals that died during the study as well as those euthanatized at the end of the 14 day observation period (Table 1). The protocol, principal personnel involved in the study, composition characteristics and Quality Assurance statement are contained in Appendices I - IV.

[a] Charles River Breeding Laboratories, Inc., Wilmington, MA
[b] Ralston Purina Laboratory Chow, Ralston Purina, St. Louis, MO
[c] Popper and Sons, Inc., New Hyde Park, NY

TABLE 1

ACUTE ORAL TOXICITY STUDY - ALBINO RATS

with S-26741, Lot 653035

Mortality, Necropsy and Body Weight Data

Dose[a] (mg/kg)	Sex	Animal Number	Individual Body Weights (gm) Test Day Number: 0	7	14	Number Dead / Number Tested	Percent Dead
80	M	5R2068	307	(1-30 min)	-	5/5	100
		5R2069	304	(1-30 min)	-		
		5R2070	273	(1-30 min)	-		
		5R2071	289	(1-30 min)	-		
		5R2072	290	(1-30 min)	-		
80	F	5R2088	246	(1-30 min)	-	5/5	100
		5R2089	229	(1-30 min)	-		
		5R2090	223	(1 hr)	-		
		5R2091	235	(1-30 min)	-		
		5R2092	236	(1 hr)	-		
60	M	5R2133	344	262	297	3/5	60
		5R2134**	289	(1 hr)	-		
		5R2135	373	(1 hr)	-		
		5R2136**	385	(1 hr)	-		
		5R2137**	294	300	323		
60	F	5R2153*	255	(1 hr)	-	3/5	60
		5R2154	215	(1 hr)	-		
		5R2155	261	269	284		
		5R2156	236	(1 hr)	-		
		5R2157	242	256	270		

TABLE 1 (concluded)

ACUTE ORAL TOXICITY STUDY - ALBINO RATS

with S-26741, Lot 653035

Mortality, Necropsy and Body Weight Data

Dose[a] (mg/kg)	Sex	Animal Number	Individual Body Weights (gm) Test Day Number: 0	7	14	Number Dead / Number Tested	Percent Dead
40	M	5R2494	209	(1 hr)	-	1/5	20
		5R2495	213	258	307		
		5R2496	208	257	302		
		5R2497	216	276	331		
		5R2498	209	251	291		
40	F	5R2514	204	235	247	1/5	20
		5R2515	201	232	243		
		5R2516	200	238	254		
		5R2517	206	251	243		
		5R2518	208	(1 hr)	-		

[a] The test article was administered as a solution in sterile distilled water.

Note: The figures in parenthesis indicate time of death.

* See necropsy section below.

** See necropsy section below.

The acute oral LD50 is 52 mg/kg with 95 confidence limits of (42-61) mg/kg in fasted albino rats.

Necropsy

A gross necropsy was performed on all rats on study. The three rats marked with (**) had hyperemic lungs and one rat marked with (*) had hemorrhage of the small intestine. All other rats on study had no visible lesions.

Table 2

ACUTE TOXICITY STUDY - ALBINO RATS
with S-26741, Lot 653035

Summary of Reactions

Dose mg/kg	Reactions / Sex	Observation Periods: Minutes 1-30	Minutes 60	Minutes 120	Number Affected/Number Dosed 1	2	3	4	5	6	7	8	9	10	11	12	13	14
80	M																	
	Hypoactivity	5/5	*															
	Prostration	5/5																
	Clonic convulsions	5/5																
	Salivation	5/5																
	Red lacrimation	5/5																
80	F																	
	Hypoactivity	5/5	2/2	*														
	Prostration	3/5	2/2															
	Clonic convulsions	5/5	2/2															
	Salivation	5/5	2/2															
	Red lacrimation	3/5	2/2															
60	M																	
	Hypoactivity	5/5	0/2		2/2	2/2	0/2											
	Ataxia	5/5	2/2	2/2	0/2													
	Salivation	5/5	0/2															
	Involuntary muscle twitching	5/5	0/2	0/2	0/2	2/2	0/2											
	Tremors		2/2	2/2	0/2													
	Urinary incontinence				2/2	2/2	0/2											
	Lethargy		2/2	2/2	0/2													
	Red lacrimation				2/2	2/2	0/2											
	Malocclusion									1/2	1/2	1/2	1/2	-	-	1/1	1/2	1/2

* Indicates total death

Table 2 (concluded)

ACUTE TOXICITY STUDY - ALBINO RATS
with S-26741, Lot 653035

Summary of Reactions

Dose mg/kg	Reactions / Sex	Minutes 1-30	Minutes 60	Minutes 120	Observation Periods Number Affected/Number Dosed 1	2	3	4	5	6	7	8	9	10	11	12	13	14
60	F																	
	Hypoactivity	5/5	2/2	2/2	0/2													
	Ataxia	5/5	2/2	0/2														
	Salivation	2/5	2/2	0/2														
	Involuntary muscle twitching	5/5		0/2														
	Tremors			2/2	0/2													
	Lethargy			2/2	0/2													
40	M																	
	Hypoactivity	5/5	4/5	4/4	0/4													
	Clonic convulsions		5/5	4/4	0/4													
	Salivation		5/5	4/4	0/4													
	Red lacrimation		5/5	4/4	0/4													
40	F																	
	Hypoactivity	5/5	4/5	4/4	0/4													
	Clonic convulsions		3/5	4/4	0/4													
	Salivation		5/5	4/4	0/4													
	Red lacrimation		5/5	4/4	0/4													

Riker Experiment No.: 0385AR0413

APPENDIX I

7.

PROTOCOL

661

TEST: **Acute Oral Toxicity**

SPONSOR: **3M** RIKER **Division**

CONDUCTED BY: **Safety Evaluation Laboratory, Riker Laboratories, Inc., St. Paul, Minnesota**

TEST ARTICLE: S-26741, Pyridostigmine Bromide, Lot 653035

CONTROL ARTICLE:

PROPOSED STARTING/COMPLETION DATE OF TEST: 9/85 - 12/85

TEST SYSTEM: **Albino Rat, CD**

SOURCE: **Charles River Breeding Laboratories, Wilmington, MA**

Sex: ♂ & ♀
Number: 5/sex/level
Weight Range: 200-300 grams

OBJECTIVE: The objective of this test will be to characterize the acute ORAL toxicity of the test article in albino rats. Rats were selected as a test system for reproducibility of response, historical use, ease in handling and general availability.

METHOD. The animals will be housed in stainless steel suspended wire mesh cages in temperature and humidity controlled rooms during both the quarantine and test periods, with food[a] and water offered *ad libitum*[b]. Each animal will be identified by color coding, according to the laboratory's standard operating procedure, which will correspond to the animal numbers on a card affixed to the outside of the cage. A single dosage of 80 mg/kg will be administered each animal, however, if this dosage level does not adequately characterize the toxicity of the test article, additional animals will be administered the test article at supplemental dosage levels. Any additional dosage levels will be documented and filed with this protocol. The test article will be administered to the animals in the form received from the sponsor. After administration of the test article, the animals will be returned to their cages and observed for any untoward behavorial reactions for the following 14 days. Initial and final body weights will be recorded. A gross necropsy which will include, but not be limited to heart, lungs, liver, kidneys and general gastrointestinal tract will be conducted on all animals which die during the conduct of the test as well as the animals surviving the test period. Any gross abnormalities which are observed during the conduct of the necropsy will be recorded with specific mention to the organ and/or site observed. The acute medial lethal dose (LD_{50}) of the test article will be calculated, if possible, using a probit analysis method at the end of the observation period. All raw data generated by the study director and the final report will be stored in the Riker Laboratories' Archive, St. Paul, Minnesota.

[a] Purina Laboratory Chow, Ralston Purina, St. Louis, Missouri
[b] Food will be withheld for a 16-20 hour period prior to dosing.

K L Eckhaus 9/18/85
Sponsor Date

Gene L. Harris 9/18/85
Study Director Date

RECEIVED
SEP 18 1985
PATHOLOGY AND TOXICOLOGY

Form 26482-1-PWO

Appendix I (concluded)
Deviations and/or Amendments to Protocol

562

1. The body weight range for this study may be 200-390 grams.

G. L. Harris 9/24/85
Study Director Date

2. Due to a technician error, final body weights were not taken on the rats in the 60 and 40 mg/kg dose levels. These two levels will be repeated so that complete results can be obtained.

G. L. Harris 11/5/85
Study Director Date

3.

Study Director Date

4.

Study Director Date

5.

Study Director Date

APPENDIX II

Principal Participating Personnel Involved in the Study

Name	Function
G. L. Harris, BS	Advanced Toxicologist Study Director
G. E. Hart	Sr. Laboratory Technician Acute Toxicology
K. D. O'Malley, BS	Senior Toxicologist Acute Toxicology
K. L. Ebbens, BS	Supervisor Toxicology Testing
G. C. Pecore	Supervisor Animal Laboratory
L. O. Wiseth	Sr. Laboratory Technician Acute Toxicology

604

APPENDIX III-A

Test and/or Control Article Characterization

for

S-26741, (PYRIDOSTIGMINE BROMIDE), LOT# 653035

1. The identity strength, uniformity, composition, purity or other pertinent characterizations of the test and/or control substances have been determined and documented as of 8/19/85.

2. The method of synthesis or origin of the test and control substances, including their amount and the method of bioassay (if applicable) is documented.

yes X no ___ ~~(NOT APPLICABLE)~~ RE 6H 1/30/8.

3. The stability of the test and/or control substances have been determined or will be determined as of ___.

Raw material stability (NOT REQUIRED FOR ACUTE STUDIES) - SEE PATHOLOGY/TK SOP's

The above information and documentation are located in the sponsor's records.

Arthur G. Rich 1/30/86

Sponsor Date

Riker St. Paul Drug Clearance Certificate

[X] Original Clearance [ ] Re-Clearance

Purpose: Reference Standard		
Sample Description: Pyridostigmine Bromide		
Compound/Lot No: Hoffman-LaRoche Lot #653035	Batch Size: 200 gm	RFA: 11055
Reference Standard Lot: -	Previous References: -	

Test Results [X] Full Clearance [ ] Selected Tests

Assay: 99.19% (on the dried basis)

Loss on Drying: 0.51%

Identification:

Infrared Spectrum: Spectrum IR 1492 agrees with USP Reference Standard Spectrum IR 1491.

Ultraviolet Spectrum: Spectrum UV 1565 agrees with USP Reference Standard Spectrum UV 1564.
Respective absorptivity 103.0% of USP Reference Standard.

Identification C: Responds to identification test.

Identification D: Responds to test for Bromide.

Melting Range: 154.2° - 155.0°

Residue on Ignition: 0

Note. Specifications or reference value in parenthesis

* Not formal clearance specification

Comments

Reference USP XXI

Analytical Review	Date	Quality Control Approval	Date
C. A. Kolars	10-14-85		9JAN86

Form 2212 Rev.

APPENDIX IV

QUALITY ASSURANCE STATEMENT

Acute Toxicology Laboratory Studies

Study No.: 0385AR0413

This short term study was audited by Compliance Audit and the final report examined against the raw data on FEBRUARY 6,1986. The results of the audit were reported to the study director and to management on FEBRUARY 6,1986.

In addition to the data audit, different significant phases for studies underway in the Acute Toxicology Laboratory are inspected weekly on a recurring cyle, and the facilities are examined by Compliance Audit on a three month schedule.

D. M. Marbet, D

Compliance Audit

2-6-86

Date

Primary Skin Irritation Test
with S-26741, Lot 653035
in Albino Rabbits

Experiment No: 0385EB0414

Conducted At: Pathology and Toxicology
Riker Laboratories, Inc.
St. Paul, Minnesota

Dates Conducted: September 24, 1985 to October 4, 1985

Conducted By: Gina L. Harris 12/5/85
G. L. Harris, BS Date
Advanced Toxicologist
Study Director

Reviewed By: [signature] 12/10/...
K. D. O'Malley, BS Date
Senior Toxicologist
Acute Toxicology

[signature] 12/11/85
K. L. Ebbens, BS Date
Supervisor, Toxicology Testing

dc: R. T. Catherall
M. W. Downing
K. L. Ebbens
A. K. Mitra
M. J. Westfall
Path/Tox Files

Summary

The results of the primary skin irritation test conducted from September 24, 1985 to October 4, 1985 at Riker Laboratories, Inc., St. Paul, Minnesota indicate that S-26741, Pyridostigmine Bromide (Lot 653035) is non-irritating (0.0/8.0) to the skin of female albino rabbits at concentrations of 50%, 15%, 5% and 1% (w/w solutions in normal saline). Each concentration was applied to one abraded and one intact test site on a group of six rabbits. Neither erythema nor edema were noted on any site during the study.

Introduction

The objective of this study was to determine the primary skin irritation potential of S-26741, Pyridostigmine Bromide (Lot 653035), to the skin of female albino rabbits. This study was conducted in accordance with the Food and Drug Administration's Good Laboratory Practice Regulation of 1978. The raw data generated by the Study Director and the final report are stored in the conducting laboratory's archives.

Animals and Husbandry

Twenty four young New Zealand White Rabbits[a] were used in the evaluation of the primary skin irritating properties of the test article. The rabbits were individually housed[b] in stainless steel cages, and food[c] and water was available ad libitum. All rabbits were individually identified with ear tags and considered to be in good health at study initiation. The rabbits were housed in a temperature and humidity controlled room. Room lighting was on a 12/12 hour light/dark cycle that was automatically timed.

Method and Results

The test procedure was modeled after that of Draize et al[d]. One day prior to the application of the test article, the hair was clipped from the back and flanks of each rabbit and two test sites selected lateral to the midline of the back approximately ten centimeters apart.

On the day of compound administration one of the two sites was abraded by making four epidermal incisions, two perpendicular to the other two, while the other test site remained intact. Concentrations of 50%, 15%, 5% and 1% (w/w solution in normal saline) were chosen and administered to each of six rabbits per dose level. The appropriate test article solution (0.5 ml) was applied to each of the test sites on each rabbit and immediately covered with two-inch square gauze patches. The patches, which were placed directly over the test sites, were secured with gauze wrap. The trunk of each animal was then wrapped with impervious plastic sheeting[e] which held the patches in position during the one day exposure period.

At the end of one day, the plastic wrappings, patches, and all residual test article were removed by washing with water. One hour and 48 hours after removal of the test article, the intact and abraded test sites were examined and scored separately for erythema and edema on a graded scale of 0 - 4.

The average irritation produced was evaluated by adding the mean scores for erythema and edema of the intact test sites one and 48 hours post removal of the test article. Similarly, the mean scores for erythema and edema of the abraded test sites were added. These two values were totaled and divided by four to obtain the mean primary irritation index.

[a] Hazleton Dutchland, Inc., Denver, PA

[b] Animals were housed in accordance with recommendations contained in DHEW Publication No. 78-23 (NIH): Revised 1978 "Guide For the Care and Use of Laboratory Animals.

[c] Purina Lab Rabbit Chow and rabbits may be offered Alfalfa Cubes for additional roughage.

[d] Draize: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965).

[e] 10 x 12 x .002 Extra Clear polyethylene sleeves, PPC Industries, Inc., Wheeling, IL

The scoring criteria for erythema and edmea are shown below.

Scoring Criteria for Skin Reactions

Reaction	Description	Score
Erythema	Barely perceptible (Edges of area not defined)	1
	Pale red in color and area definable	2
	Definite red in color and area well defined.	3
	Beet or crimson red in color	4
Edema	Barely perceptible (Edges of area not defined)	1
	Area definable but not raised more than 1 mm.	2
	Area well defined and raised approximately 1 mm.	3
	Area raised more than 1 mm.	4
	Maximum Primary Irritation Score =	8

The following grading system was used to arrive at a descriptive primary skin irritation rating:

Mean Primary Irritation Score (Range of Values)	Descriptive Rating
0	Non-Irritating
0.1 - 0.5	Minimally Irritating
0.6 - 1.5	Slightly Irritating
1.6 - 3.0	Mildly Irritating
3.1 - 5.0	Moderately Irritating
5.1 - 6.5	Severely Irritating
6.6 - 8.0	Extremely Irritating

The rating for a test article may be increased if the reactions caused are beyond simple erythema and edema, e.g. necrosis, escharosis, hemorrhage. The results are presented in Tables 1, 2, 3, and 4. The protocol, principal personnel involved in the study, composition characteristics and Quality Assurance statement are contained in Appendices I - IV.

Table 1

Primary Skin Irritation Test - Albino Rabbits

with S-26741
(50% w/w/ solution in saline)

Animal Number	Irritation Scores for Abraded Skin Sites after Removal: 1 Hour Er.	1 Hour Ed.	48 Hours Er.	48 Hours Ed.	Irritation Scores for Intact Skin Sites after Removal: 1 Hour Er.	1 Hour Ed.	48 Hours Er.	48 Hours Ed.
5B1506	0	0	0	0	0	0	0	0
5B1509	0	0	0	0	0	0	0	0
5B1501	0	0	0	0	0	0	0	0
5B1504	0	0	0	0	0	0	0	0
5B1507	0	0	0	0	0	0	0	0
5B1510	0	0	0	0	0	0	0	0
Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Subtotal			0.0				0.0	

Rating: Non-irritating

Primary Irritation Index: 0.0/8.0

Key: Er. = Erythema
Ed. = Edema

Table 2

Primary Skin Irritation Test - Albino Rabbits

with S-26741
(15% w/w solution in saline)

Animal Number	Irritation Scores for Abraded Skin Sites after Removal: 1 Hour Er.	1 Hour Ed.	48 Hours Er.	48 Hours Ed.	Irritation Scores for Intact Skin Sites after Removal: 1 Hour Er.	1 Hour Ed.	48 Hours Er.	48 Hours Ed.
5B1434	0	0	0	0	0	0	0	0
5B1437	0	0	0	0	0	0	0	0
5B1469	0	0	0	0	0	0	0	0
5B1443	0	0	0	0	0	0	0	0
5B1435	0	0	0	0	0	0	0	0
5B1438	0	0	0	0	0	0	0	0
Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Subtotal			0.0				0.0	

Rating: Non-irritating

Primary Irritation Index: 0.0/8.0

Key: Er. = Erythema
Ed. = Edema

Table 3

Primary Skin Irritation Test - Albino Rabbits

with S-26741
(5% w/w solution in saline)

Animal Number	Irritation Scores for Abraded Skin Sites after Removal: 1 Hour Er.	1 Hour Ed.	48 Hours Er.	48 Hours Ed.	Irritation Scores for Intact Skin Sites after Removal: 1 Hour Er.	1 Hour Ed.	48 Hours Er.	48 Hours Ed.
5B1441	0	0	0	0	0	0	0	0
5B1444	0	0	0	0	0	0	0	0
5B1436	0	0	0	0	0	0	0	0
5B1466	0	0	0	0	0	0	0	0
5B1442	0	0	0	0	0	0	0	0
5B1445	0	0	0	0	0	0	0	0
Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Subtotal			0.0				0.0	

Rating: Non-irritating

Primary Irritation Index: 0.0/8.0

Key: Er. = Erythema
Ed. = Edema

Table 1

Primary Skin Irritation Test - Albino Rabbits

with S-26741
(1% w/w solution in saline)

Animal Number	Irritation Scores for Abraded Skin Sites after Removal: 1 Hour Er.	1 Hour Ed.	48 Hours Er.	48 Hours Ed.	Irritation Scores for Intact Skin Sites after Removal: 1 Hour Er.	1 Hour Ed.	48 Hours Er.	48 Hours Ed.
5B1446	0	0	0	0	0	0	0	0
5B1449	0	0	0	0	0	0	0	0
5B1452	0	0	0	0	0	0	0	0
5B1455	0	0	0	0	0	0	0	0
5B1447	0	0	0	0	0	0	0	0
5B1465	0	0	0	0	0	0	0	0
Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Subtotal			0.0				0.0	

Rating: Non-irritating

Primary Irritation Index: 0.0/8.0

Key: Er. = Erythema
Ed. = Edema

APPENDIX I Riker Experiment No.: 0585EB0414

PROTOCOL

TEST: Acute Primary Skin Irritation Test 515

SPONSOR: 3M Riker Division

CONDUCTED BY: **Safety Evaluation Laboratory, Riker Laboratories, Inc., St. Paul, Minnesota**

TEST ARTICLE: S-26741, Pyridostigmine Bromide, Lot 653035

CONTROL ARTICLE: ———

PROPOSED STARTING/COMPLETION DATE OF TEST: 9/85 - 12/85

TEST SYSTEM: Female New Zealand White Albino Rabbits

SOURCE: Hazelton Dutchland
Denver, PA

OBJECTIVE. To determine the irritation potential of the test article to the skin of 18 animals. Rabbits were selected as the test system due to their historical use, sensitivity to irritants, ease of handling and general availability.

METHOD. The animals will be housed in standard wire-mesh cages in temperature and humidity controlled rooms with food[a] and water offered *ad libitum*. Each animal will be assigned a numbered ear tag, which will correspond to a card affixed to the outside of the cage. Prior to the application of the test article, the hair will be clipped from the back and flanks of each animal and 2 test sites selected lateral to the midline of the back approximately ten centimeters apart. 1 of the 2 sites will be abraded by making four epidermal incisions, two perpendicular to the other two, while the other test site(s) will remain intact. The test article (0.5 ml *) will be applied to 1 abraded and 1 intact site(s) of each animal. The trunk of each animal will then be wrapped with impervious plastic sheeting which will occlude the test article during the 1 day exposure period One hour and 48 hours after removal of the test article, the intact and abraded test sites will be examined and scored separately for erythema and edema on a graded scale of 0 to 4[b]. The average irritation produced will be evaluated by adding the mean scores for erythema and edema of the intact test sites one and 48 hours post removal of the test article. Similarly, the mean scores for erythema and edema of the abraded test sites will be added. These two values will be totaled and divided by four to obtain the mean primary irritation index and then assigned a descriptive primary skin irritation rating as follows:

Mean Primary Irritation Score	Descriptive Rating
0	Non-irritating
0.1 - 0.5	Minimally Irritating
0.6 - 1.5	Slightly Irritating
1.6 - 3.0	Mildly Irritating
3.1 - 5.0	Moderately Irritating
5.1 - 6.5	Severely Irritating
6.6 - 8.0	Extremely Irritating

The rating for a test article may be increased if the reaction caused is beyond erythema and edema and are deemed to be of importance in the interpretation of the results. All raw data generated by the study director and the final report will be stored in the Riker Laboratories Archives, St Paul, Minnesota

[a] Purina Rabbit Chow, Ralston Purina Co., St. Louis, Missouri
[b] Draize: Appraisal of the Safety of Chemicals in Food, Drugs and Cosmetics (1965) Published by the Editorial Committee of the Association of Food and Drug Officials of the United States

*=Three groups of 6 animals each will be treated respectively 0.5 ml of a 15%, 5% and 1% w/w concentration of S-26741 in saline. GH 9/13/85

K. Eckhaus — Sponsor — Date

Jane L Harris 9/13/85 — Study Director — Date

Form 19171-8-A-PWO

Appendix I (concluded)
Deviations and/or Amendments to Protocol

1. An additional six animals will be dosed with a 50% w/w solution of S-26741 in saline.

G. L. Harris — Study Director — 9/27/85 — Date

2. The test material was secured to the rabbit back with gauze during the study.

G. L. Harris — Study Director — 1/9/86 — Date

3. The sponsor did not date the protocol at the time of signature. This date should be 9/18/85 as it was signed by the sponsor and study director on the same day.

G. L. Harris — Study Director — 1/9/86 — Date

4. _____

Study Director — Date

5. _____

Study Director — Date

APPENDIX III - A

Test and/or Control Article Characterization

for

S-26741, (PYRIDOSTIGMINE BROMIDE), LOT# 6530S

1. The identity strength, uniformity, composition, purity or other pertinent characterizations of the test and/or control substances have been determined and documented as of 8/19/85.

2. The method of synthesis or origin of the test and control substances, including their amount and the method of bioassay (if applicable) is documented.

yes X no ___ ~~(NOT Applicable)~~ RE. EH 1/30/

3. The stability of the test and/or control substances have been determined or will be determined as of ___.

Raw material stability (NOT REQUIRED FOR ACUTE STUDIES) - See PATHOL. SOP

The above information and documentation are located in the sponsor's records.

Amit G. Datta 1/30/86

Sponsor Date

Riker St. Paul Drug Clearance Certificate

3M

☒ Original Clearance ☐ Re-Clearance

Purpose: Reference Standard		
Sample Description: Pyridostigmine Bromide		
Compound/Lot No: Hoffman-LaRoche Lot #653035	Batch Size: 200 gm	RFA: 11055
Reference Standard Lot: -	Previous References: -	

Test Results ☒ Full Clearance ☐ Selected Tests

Assay: 99.19% (on the dried basis)

Loss on Drying: 0.51%

Identification:

Infrared Spectrum: Spectrum IR 1492 agrees with USP Reference Standard Spectrum IR 1491.

Ultraviolet Spectrum: Spectrum UV 1565 agrees with USP Reference Standard Spectrum UV 1564.
Respective absorptivity 103.0% of USP Reference Standard.

Identification C: Responds to identification test.

Identification D: Responds to test for Bromide.

Melting Range: 154.2° - 155.0°

Residue on Ignition: 0

Note Specifications or reference value in parenthesis

* Not formal clearance specification

Comments

Reference USP XXI

Analytical Review	Date	Quality Control Approval	Date
C. A. Kolars	10-14-85		9JAN8

Form 14482 8-PWO

RFA – 11055 | 1 of 1

Submitted By	Telephone No.	Department No.	Date
A. K. Wiltrout		9197	8-19-85

Address	Notebook No.	Project	Project No.
		902.10007	

Description of Sample (Write a complete description of the sample such as structural formula, compound and lot number, source, reaction schematic, solubility, mp, bp, mol. wt., etc.)

PYRIDOSTIGMINE BROMIDE 619

HOFFMAN-LAROCHE LOT # 653035

Special Precautions - HYGROSCOPIC

Work Desired

CLEARANCE VS USP XXI SPECIFICATIONS

RUN RP LIGHT TRANSMISSION & TLC TESTS

Amount Available	Sample Disposal	Received By
200 g	☐ Return ☐ Discard ☑ Retain	

Results

USP NB # 55 pg 15-22, 23

ID – A: IR Spectra Pass attached Jeff M. Zumbyle 9-5-85

B: UV Spectra Pass attached

C: Pyridostigmine TD Pass Charles K. Lee 5-7-85

D: Bromide Pass

Melting Range – Limit 154°-157° – Actual 154.2°-155.0°

LOD – not more than 2.0% – Actual 0.51%

ROI – Limit not more than 0.1% – Actual 0%

Assay – nonaqueous titration – Limit 98.5-100.5% – Actual 99.2% on dried basis

BP – Light Absorption – Limit – not more than 1.0 – Actual .037

Related Substances (TLC) Pass

BP Limit tests inconclusive – Results do not approach limits

APPENDIX IV

QUALITY ASSURANCE STATEMENT

Acute Toxicology Laboratory Studies

Study No.: 0385EB0414

This short term study was audited by Compliance Audit and the final report examined against the raw data on 12/18/85. The results of the audit were reported to the study director and to management on 12/18/85.

In addition to the data audit, different significant phases for studies underway in the Acute Toxicology Laboratory are inspected weekly on a recurring cyle, and the facilities are examined by Compliance Audit on a three month schedule.

J. A. McCauley
Compliance Audit

12/18/85
Date

Riker Experiment No.: 0385EB0414

REPORT ADDENDUM

621

1. To meet G.L.P. requirements, Appendix III - A and III - B are being added to the final report. The original Appendix III is now changed to read Appendix III-C.

G. L. Harris 2/7/86
Study Director Date

2. _____

Study Director Date

3. _____

Study Director Date

4. _____

Study Director Date

5. _____

Study Director Date

Pathology and Toxicology
Riker Laboratories, Inc.

Building 270-3S-05, 3M Center
St. Paul, Minnesota 55144-1000

3M

Primary Skin Irritation Test

with 0.35% Docusate Sodium (w/v solution in water and 5% glycerin)

in Albino Rabbits

Riker Experiment No:	0386EB0576
Conducted At:	Pathology and Toxicology Riker Laboratories, Inc.
Dates Conducted:	October 14, 1986 to October 17, 1986

Conducted By:

Gina L. Harris 11/7/86

G. L. Harris, B.S. Date
Advanced Toxicologist
Study Director and Acute Toxicity
Study Coordinator

Reviewed By:

J. Allen 11-11-86

J. L. Allen, Ph.D. Date
Diplomate, A.B.T.
Research Toxicologist

dc: J. L. Allen
R. T. Catherall
C. F. Chesney
M. W. Downing
N. M. Marecki
M. J. Westfall
Tech. Doc. Cntr.
Path/Tox Files

Summary

The results of the primary skin irritation test conducted from October 14, 1986 to October 17, 1986 at Riker Laboratories, Inc., St. Paul, Minnesota indicate that 0.35% Docusate Sodium (w/v solution in water and 5% glycerin) is non-irritating (0.0/8.0) to the skin of female albino rabbits. Neither erythema nor edema were noted at any time during the study.

Introduction

The objective of this study was to determine the primary skin irritation potential of 0.35% Docusate Sodium (w/v solution in water and 5% glycerin) to the skin of female albino rabbits. This study was conducted for research and development purposes and is, therefore, not regulated by the Food and Drug Administration's Good Laboratory Practice Regulation of 1978, although the standard operating procedures of this laboratory adhere to the general principles of this regulation. The raw data generated by the Study Director and the final report are stored in the conducting laboratory's archives.

Animals and Husbandry

Young New Zealand White Rabbits[a] were used in the evaluation of the primary skin irritating properties of the test article. The rabbits were individually housed[b] in stainless steel cages, and food[c] and water were available ad libitum. All rabbits were individually identified with ear tags and considered to be in good health at study initiation. The rabbits were housed in a temperature and humidity controlled room. Room lighting was on a 12/12 hour light/dark cycle that was automatically timed.

Method and Results

The test procedure was modeled after that of Draize et al[d]. One day prior to the application of the test article, the hair was clipped from the back and flanks of each rabbit and two test sites selected lateral to the midline of the back approximately ten centimeters apart.

On the day of compound administration one of the two sites was abraded by making four epidermal incisions, two perpendicular to the other two, while the other test site remained intact. The test article (0.5 ml) was applied to each of the test sites on each rabbit and immediately covered with two-inch square gauze patches. The patches, which were placed directly over the test sites, were secured with gauze wrap. The trunk of each animal was then wrapped with impervious plastic sheeting[e] which held the patches in position during the one day exposure period.

At the end of one day, the plastic wrappings, patches, and all residual test article were removed by washing with water. One hour and two days after removal of the test article, the intact and abraded test sites were examined and scored separately for erythema and edema on a graded scale of 0 - 4.

The average irritation produced was evaluated by adding the mean scores for erythema and edema of the intact test sites one hour and two days post removal of the test article. Similarly, the mean scores for erythema and edema of the abraded test sites were added. These two values were totaled and divided by four to obtain the mean primary irritation index.

[a] Hazleton Dutchland, Inc., Denver, PA
[b] Animals were housed in accordance with recommendations contained in DHEW Publication No. 78-23 (NIH): Revised 1978 "Guide For the Care and Use of Laboratory Animals.
[c] Purina Lab Rabbit Chow® and rabbits may be offered Alfalfa Cubes for additional roughage.
[d] Draize: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965).
[e] 10 x 12 x .002 Extra Clear polyethylene sleeves, PPC Industries, Inc., Wheeling, IL

The scoring criteria for erythema and edema are shown below.

Scoring Criteria for Skin Reactions

Reaction	Description	Score
Erythema	Barely perceptible (Edges of area not defined)	1
	Pale red in color and area definable	2
	Definite red in color and area well defined.	3
	Beet or crimson red in color	4
Edema	Barely perceptible (Edges of area not defined)	1
	Area definable but not raised more than 1 mm.	2
	Area well defined and raised approximately 1 mm.	3
	Area raised more than 1 mm.	4
	Maximum Primary Irritation Score =	8

The following grading system was used to arrive at a descriptive primary skin irritation rating:

Mean Primary Irritation Score (Range of Values)	Descriptive Rating
0	Non-Irritating
0.1 - 0.5	Minimally Irritating
0.6 - 1.5	Slightly Irritating
1.6 - 3.0	Mildly Irritating
3.1 - 5.0	Moderately Irritating
5.1 - 6.5	Severely Irritating
6.6 - 8.0	Extremely Irritating

The rating for a test article may be increased if the reactions caused are beyond simple erythema and edema, e.g. necrosis, escharosis, hemorrhage. The results are presented in Table 1. The protocol, principal personnel involved in the study, composition characteristics and Quality Assurance statement are contained in Appendices I - IV.

Table 1

Primary Skin Irritation Test - Albino Rabbits

with 0.35% Docusate Sodium
(w/v solution in water and 5% glycerin)

Animal Number	Irritation Scores for Abraded Skin Sites after Removal: 1 Hour Er.	1 Hour Ed.	Day 2 Er.	Day 2 Ed.	Irritation Scores for Intact Skin Sites after Removal: 1 Hour Er.	1 Hour Ed.	Day 2 Er.	Day 2 Ed.
6B1597	0	0	0	0	0	0	0	0
6B1600	0	0	0	0	0	0	0	0
6B1603	0	0	0	0	0	0	0	0
6B1606	0	0	0	0	0	0	0	0
6B1598	0	0	0	0	0	0	0	0
6B1601	0	0	0	0	0	0	0	0
Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Subtotal		0.0				0.0		

Rating: Non-irritating

Primary Irritation Index: 0.0/8.0

Key: Er. = Erythema
Ed. = Edema

APPENDIX I Riker Experiment No: 0386EB0576

PROTOCOL

627

TEST: Acute Primary Skin Irritation Test

SPONSOR: 3M RIKER Division

CONDUCTED BY: Pathology and Toxicology Department, Riker Laboratories, Inc. St. Paul, Minnesota

TEST ARTICLE: 0.35% Docusate Sodium (w/v solution in water & 5% glycerin)

CONTROL ARTICLE: NONE

PROPOSED STARTING/COMPLETION DATE OF TEST: 10/86 – 12/86

TEST SYSTEM: New Zealand White Albino Rabbits (of either sex)

SOURCE: Hazleton Dutchland, Inc., Denver, Pa.

OBJECTIVE: To determine the primary irritation potential of the test article to the skin of 6 animals. Rabbits were selected as the test system due to their historical use, sensitivity to irritants, ease of handling and general availability.

METHOD: The animals will be housed in standard wire-mesh cages in temperature and humidity controlled rooms and food[a] and water offered *ad libitum*. Each animal will be assigned a numbered ear tag, which will correspond to a card affixed to the outside of cage. Prior to the application of the test article, the hair will be clipped from the back and flanks of each animal and 2 test sites selected lateral to the midline of the back approximately ten centimeters apart. 1 of the 2 sites will be abraded by making four epidermal incisions two perpendicular to the other two, while the other test site(s) will remain intact. The test article (0.5 ml) will be applied to 1 abraded and 1 intact site(s) on each animal and secured. The trunk of each animal will then be wrapped with impervous plastic sheeting which will occlude the test article during the 1 day exposure period. One hour and 2 days after removal of the test article during the 1 day exposure period. One hour and 2 days after removal of the test article, the intact and abraded test sites will be examined and scored separately for erythema and edema on a graded scale of 0 to 4. The average irritation produced will be evaluated by adding the mean scores for erythema and edema of the intact test sites one hour and 2 days post removal of the test article. Similarly, the mean scores for erythema and edema of the abraded test sites will be added. These two values will be totaled and divided by four to obtain the mean primary irritation index and then assigned a descriptive primary skin irritation rating as follows:

Mean Primary Irritation Score	Descriptive Rating
0	Non-irritating
0.1 - 0.5	Minimally Irritating
0.6 - 1.5	Slightly Irritating
1.6 - 3.0	Midly Irritating
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5.1 - 6.5	Severely Irritating
6.6 - 8.0	Extremely Irritating

The rating for a test article may be increased if the reaction caused is beyond erythema and edema and are deemed to be of importance in the interpretation of the results. All raw data generated by the study director and the final report will be stored in the Riker Laboratories' Archive, St. Paul, Minnesota.

[a] Purina Rabbit Chow, Ralston Purina Co., St. Louis, Missouri

[b] Draize. Apprasisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965) Published by the Editorial Committee of the Association of Food and Drug Officials of the United States.

Sponsor 10/10/86 Date

Study Director 10/10/86 Date

Form 19171 - 12 - F - PWO

APPENDIX II

Principal Participating Personnel Involved in the Study

Name	Function
G. L. Harris, B.S.	Advanced Toxicologist Study Director
G. E. Hart	Master Laboratory Technician Acute Toxicology
L. O. Wiseth	Sr. Laboratory Technician Acute Toxicology
J. A. Eads	Jr. Laboratory Technician Acute Toxicology
J. L. Allen, Ph.D.	Research Toxicologist Acute Toxicology
C. C. Pecore	Supervisor Animal Laboratory

APPENDIX III

Composition Characteristics

This study is not regulated by the Good Laboratory Practice Act of 1978 and therefore information pertaining to composition characteristics is not applicable for inclusion in this study.

APPENDIX IV

Quality Assurance Statement

This study is not officially regulated by the Good Laboratory Practice Regulation of 1978, and therefore a statement signed and prepared by the Compliance Audit department is not applicable.

The standard operating procedures of this laboratory does adhere to the general principles of this regulation. The Compliance Audit department does inspect differenct significant phases for studies underway in the Acute Toxicology Laboratory on a recurring cycle, and the facilities are examined on a three month schedule. In addition a select number of Research & Development studies are routinely picked at random from the Archives by the Compliance Audit department for review.

Pathology and Toxicology
Riker Laboratories, Inc.

Building 270-3S-05, 3M Center
St. Paul, Minnesota 55144-1000

3M

Primary Skin Irritation Test

with 0.33% Sodium Lauryl Sulfate (w/v solution in water and 5% glycerin)

in Albino Rabbits

Riker Experiment No: 0386EB0577

Conducted At: Pathology and Toxicology
Riker Laboratories, Inc.

Dates Conducted: October 14, 1986 to October 17, 1986

Conducted By: Gene L. Harris 11/7/86
G. L. Harris, B.S. Date
Advanced Toxicologist
Study Director and Acute Toxicity
Study Coordinator

Reviewed By: J. Allen 11-11-86
J. L. Allen, Ph.D. Date
Diplomate, A.B.T.
Research Toxicologist

dc: J. L. Allen
R. T. Catherall
C. F. Chesney
M. W. Downing
N. M. Marecki
M. J. Westfall
Tech. Doc. Cntr.
Path/Tox Files

Summary

The results of the primary skin irritation test conducted from October 14, 1986 to October 17, 1986 at Riker Laboratories, Inc., St. Paul, Minnesota indicate that 0.33% Sodium Lauryl Sulfate (w/v solution in water and 5% glycerin) is non-irritating (0.0/8.0) to the skin of female albino rabbits. Neither erythema nor edema were noted at any time during the study.

Introduction

The objective of this study was to determine the primary skin irritation potential of 0.33% Sodium Lauryl Sulfate (w/v solution in water and 5% glycerin) to the skin of female albino rabbits. This study was conducted for research and development purposes and is, therefore, not regulated by the Food and Drug Administration's Good Laboratory Practice Regulation of 1978, although the standard operating procedures of this laboratory adhere to the general principles of this regulation. The raw data generated by the Study Director and the final report are stored in the conducting laboratory's archives.

Animals and Husbandry

Young New Zealand White Rabbits[a] were used in the evaluation of the primary skin irritating properties of the test article. The rabbits were individually housed[b] in stainless steel cages, and food[c] and water were available ad libitum. All rabbits were individually identified with ear tags and considered to be in good health at study initiation. The rabbits were housed in a temperature and humidity controlled room. Room lighting was on a 12/12 hour light/dark cycle that was automatically timed.

Method and Results

The test procedure was modeled after that of Draize et al[d]. One day prior to the application of the test article, the hair was clipped from the back and flanks of each rabbit and two test sites selected lateral to the midline of the back approximately ten centimeters apart.

On the day of compound administration one of the two sites was abraded by making four epidermal incisions, two perpendicular to the other two, while the other test site remained intact. The test article (0.5 ml) was applied to each of the test sites on each rabbit and immediately covered with two-inch square gauze patches. The patches, which were placed directly over the test sites, were secured with gauze wrap. The trunk of each animal was then wrapped with impervious plastic sheeting[e] which held the patches in position during the one day exposure period.

At the end of one day, the plastic wrappings, patches, and all residual test article were removed by washing with water. One hour and two days after removal of the test article, the intact and abraded test sites were examined and scored separately for erythema and edema on a graded scale of 0 - 4.

The average irritation produced was evaluated by adding the mean scores for erythema and edema of the intact test sites one hour and two days post removal of the test article. Similarly, the mean scores for erythema and edema of the abraded test sites were added. These two values were totaled and divided by four to obtain the mean primary irritation index.

[a] Hazleton Dutchland, Inc., Denver, PA
[b] Animals were housed in accordance with recommendations contained in DHEW Publication No. 78-23 (NIH): Revised 1978 "Guide For the Care and Use of Laboratory Animals.
[c] Purina Lab Rabbit Chow® and rabbits may be offered Alfalfa Cubes for additional roughage.
[d] Draize: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965).
[e] 10 x 12 x .002 Extra Clear polyethylene sleeves, PPC Industries, Inc., Wheeling, IL

The scoring criteria for erythema and edema are shown below.

Scoring Criteria for Skin Reactions

Reaction	Description	Score
Erythema	Barely perceptible (Edges of area not defined)	1
	Pale red in color and area definable	2
	Definite red in color and area well defined.	3
	Beet or crimson red in color	4
Edema	Barely perceptible (Edges of area not defined)	1
	Area definable but not raised more than 1 mm.	2
	Area well defined and raised approximately 1 mm.	3
	Area raised more than 1 mm.	4
	Maximum Primary Irritation Score =	8

The following grading system was used to arrive at a descriptive primary skin irritation rating:

Mean Primary Irritation Score (Range of Values)	Descriptive Rating
0	Non-Irritating
0.1 - 0.5	Minimally Irritating
0.6 - 1.5	Slightly Irritating
1.6 - 3.0	Mildly Irritating
3.1 - 5.0	Moderately Irritating
5.1 - 6.5	Severely Irritating
6.6 - 8.0	Extremely Irritating

The rating for a test article may be increased if the reactions caused are beyond simple erythema and edema, e.g. necrosis, escharosis, hemorrhage. The results are presented in Table 1. The protocol, principal personnel involved in the study, composition characteristics and Quality Assurance statement are contained in Appendices I - IV.

Table 1

Primary Skin Irritation Test - Albino Rabbits

with 0.33% Sodium Lauryl Sulfate
(w/v solution in water and 5% glycerin)

Animal Number	Irritation Scores for Abraded Skin Sites after Removal: 1 Hour Er.	1 Hour Ed.	Day 2 Er.	Day 2 Ed.	Irritation Scores for Intact Skin Sites after Removal: 1 Hour Er.	1 Hour Ed.	Day 2 Er.	Day 2 Ed.
6B1628	0	0	0	0	0	0	0	0
6B1631	0	0	0	0	0	0	0	0
6B1623	0	0	0	0	0	0	0	0
6B1626	0	0	0	0	0	0	0	0
6B1629	0	0	0	0	0	0	0	0
6B1632	0	0	0	0	0	0	0	0
Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Subtotal		0.09					0.0	

Rating: Non-irritating

Primary Irritation Index: 0.0/8.0

Key: Er. = Erythema
Ed. = Edema

APPENDIX I Riker Experiment No: 0386EB0577

PROTOCOL

5.

TEST: Acute Primary Skin Irritation Test 635

SPONSOR: 3M Riker Division

CONDUCTED BY: Pathology and Toxicology Department, Riker Laboratories, Inc. St. Paul, Minnesota

TEST ARTICLE: 0.33% Sodium Lauryl Sulfate (w/v solution with water & 5% Glycerin)

CONTROL ARTICLE: NONE

PROPOSED STARTING/COMPLETION DATE OF TEST: 10/86 - 12/86

TEST SYSTEM: New Zealand White Albino Rabbits (of either sex)

SOURCE: Hazleton Dutchland, Inc., Denver, Pa.

OBJECTIVE: To determine the primary irritation potential of the test article to the skin of 6 animals. Rabbits were selected as the test system due to their historical use, sensitivity to irritants, ease of handling and general availability.

METHOD. The animals will be housed in standard wire-mesh cages in temperature and humidity controlled rooms and food[a] and water offered *ad libitum.* Each animal will be assigned a numbered ear tag, which will correspond to a card affixed to the outside of cage. Prior to the application of the test article, the hair will be clipped from the back and flanks of each animal and 2 test sites selected lateral to the midline of the back approximately ten centimeters apart. 1 of the 2 sites will be abraded by making four epidermal incisions two perpendicular to the other two, while the other test site(s) will remain intact. The test article (0.5 mL) will be applied to 1 abraded and 1 intact site(s) on each animal and secured. The trunk of each animal will then be wrapped with impervous plastic sheeting which will occlude the test article during the 1 day exposure period. One hour and 2 days after removal of the test article during the 1 day exposure period. One hour and 2 days after removal of the test article, the intact and abraded test sites will be examined and scored separately for erythema and edema on a graded scale of 0 to 4. The average irritation produced will be evaluated by adding the mean scores for erythema and edema of the intact test sites one hour and 2 days post removal of the test article. Similarly, the mean scores for erythema and edema of the abraded test sites will be added. These two values will be totaled and divided by four to obtain the mean primary irritation index and then assigned a descriptive primary skin irritation rating as follows:

Mean Primary Irritation Score	Descriptive Rating
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The rating for a test article may be increased if the reaction caused is beyond erythema and edema and are deemed to be of importance in the interpretation of the results. All raw data generated by the study director and the final report will be stored in the Riker Laboratories' Archive, St. Paul, Minnesota.

[a] Purina Rabbit Chow, Ralston Purina Co., St. Louis, Missouri

[b] Draize: Apprasisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965) Published by the Editorial Committee of the Association of Food and Drug Officials of the United States.

Sponsor 10/10/86 Date

Study Director 10/10/86 Date

PATHOLOGY

Form 19171 12 F PWO

APPENDIX II

Principal Participating Personnel Involved in the Study

Name	Function
G. L. Harris, B.S.	Advanced Toxicologist Study Director
G. E. Hart	Master Laboratory Technician Acute Toxicology
L. O. Wiseth	Sr. Laboratory Technician Acute Toxicology
J. A. Eads	Jr. Laboratory Technician Acute Toxicology
J. L. Allen, Ph.D.	Research Toxicologist Acute Toxicology
G. C. Pecore	Supervisor Animal Laboratory

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The standard operating procedures of this laboratory does adhere to the general principles of this regulation. The Compliance Audit department does inspect differenct significant phases for studies underway in the Acute Toxicology Laboratory on a recurring cycle, and the facilities are examined on a three month schedule. In addition a select number of Research & Development studies are routinely picked at random from the Archives by the Compliance Audit department for review.

Pathology and Toxicology
Riker Laboratories, Inc.

Building 270-3S-05, 3M Center
St. Paul, Minnesota 55144-1000

640

3M

Primary Skin Irritation Test

with 1% Potassium Laurate
(w/v solution in water and 5% glycerin)

in Albino Rabbits

Riker Experiment No: 0386EB0578

Conducted At: Pathology and Toxicology
Riker Laboratories, Inc.

Dates Conducted: October 14, 1986 to October 17, 1986

Conducted By: Jane L. Harris 11/7/86
G. L. Harris, B.S. Date
Advanced Toxicologist
Study Director and Acute Toxicity
Study Coordinator

Reviewed By: J L Allen 11/11/86
J. L. Allen, Ph.D. Date
Diplomate, A.B.T.
Research Toxicologist

dc: J. L. Allen
R. T. Catherall
C. F. Chesney
M. W. Downing
N. M. Marecki
M. J. Westfall
Tech. Doc. Cntr
Path/Tox Files

Summary

The results of the primary skin irritation test conducted from October 14, 1986 to October 17, 1986 at Riker Laboratories, Inc., St. Paul, Minnesota indicate that 1% Potassium Laurate (w/v solution in water and 5% glycerin) is non-irritating (0.0/8.0) to the skin of female albino rabbits. Neither erythema nor edema were noted at any time during the study.

Introduction

The objective of this study was to determine the primary skin irritation potential of 1% Potassium Laurate (w/v solution in water and 5% glycerin) to the skin of female albino rabbits. This study was conducted for research and development purposes and is, therefore, not regulated by the Food and Drug Administration's Good Laboratory Practice Regulation of 1978, although the standard operating procedures of this laboratory adhere to the general principles of this regulation. The raw data generated by the Study Director and the final report are stored in the conducting laboratory's archives.

Animals and Husbandry

Young New Zealand White Rabbits[a] were used in the evaluation of the primary skin irritating properties of the test article. The rabbits were individually housed[b] in stainless steel cages, and food[c] and water were available ad libitum. All rabbits were individually identified with ear tags and considered to be in good health at study initiation. The rabbits were housed in a temperature and humidity controlled room. Room lighting was on a 12/12 hour light/dark cycle that was automatically timed.

Method and Results

The test procedure was modeled after that of Draize et al[d]. One day prior to the application of the test article, the hair was clipped from the back and flanks of each rabbit and two test sites selected lateral to the midline of the back approximately ten centimeters apart.

On the day of compound administration one of the two sites was abraded by making four epidermal incisions, two perpendicular to the other two, while the other test site remained intact. The test article (0.5 ml) was applied to each of the test sites on each rabbit and immediately covered with two-inch square gauze patches. The patches, which were placed directly over the test sites, were secured with gauze wrap. The trunk of each animal was then wrapped with impervious plastic sheeting[e] which held the patches in position during the one day exposure period.

At the end of one day, the plastic wrappings, patches, and all residual test article were removed by washing with water. One hour and two days after removal of the test article, the intact and abraded test sites were examined and scored separately for erythema and edema on a graded scale of 0 - 4.

The average irritation produced was evaluated by adding the mean scores for erythema and edema of the intact test sites one hour and two days post removal of the test article. Similarly, the mean scores for erythema and edema of the abraded test sites were added. These two values were totaled and divided by four to obtain the mean primary irritation index.

[a] Hazleton Dutchland, Inc., Denver, PA
[b] Animals were housed in accordance with recommendations contained in DHEW Publication No. 78-23 (NIH): Revised 1978 "Guide For the Care and Use of Laboratory Animals.
[c] Purina Lab Rabbit Chow® and rabbits may be offered Alfalfa Cubes for additional roughage.
[d] Draize: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965).
[e] 10 x 12 x .002 Extra Clear polyethylene sleeves, PPC Industries, Inc., Wheeling, IL

The scoring criteria for erythema and edema are shown below.

Scoring Criteria for Skin Reactions

Reaction	Description	Score
Erythema	Barely perceptible (Edges of area not defined)	1
	Pale red in color and area definable	2
	Definite red in color and area well defined.	3
	Beet or crimson red in color	4
Edema	Barely perceptible (Edges of area not defined)	1
	Area definable but not raised more than 1 mm.	2
	Area well defined and raised approximately 1 mm.	3
	Area raised more than 1 mm.	4
	Maximum Primary Irritation Score =	8

The following grading system was used to arrive at a descriptive primary skin irritation rating:

Mean Primary Irritation Score (Range of Values)	Descriptive Rating
0	Non-Irritating
0.1 - 0.5	Minimally Irritating
0.6 - 1.5	Slightly Irritating
1.6 - 3.0	Mildly Irritating
3.1 - 5.0	Moderately Irritating
5.1 - 6.5	Severely Irritating
6.6 - 8.0	Extremely Irritating

The rating for a test article may be increased if the reactions caused are beyond simple erythema and edema, e.g. necrosis, escharosis, hemorrhage. The results are presented in Table 1. The protocol, principal personnel involved in the study, composition characteristics and Quality Assurance statement are contained in Appendices I - IV.

Table 1

Primary Skin Irritation Test - Albino Rabbits

with 1% Potassium Laurate
(w/v solution in water and 5% glycerin)

Animal Number	Irritation Scores for Abraded Skin Sites after Removal: 1 Hour Er.	1 Hour Ed.	Day 2 Er.	Day 2 Ed.	Irritation Scores for Intact Skin Sites after Removal: 1 Hour Er.	1 Hour Ed.	Day 2 Er.	Day 2 Ed.
6B1621	0	0	0	0	0	0	0	0
6B1624	0	0	0	0	0	0	0	0
6B1627	0	0	0	0	0	0	0	0
6B1630	0	0	0	0	0	0	0	0
6B1622	0	0	0	0	0	0	0	0
6B1625	0	0	0	0	0	0	0	0
Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Subtotal		0.0				0.0		

Rating: Non-irritating

Primary Irritation Index: 0.0/8.0

Key: Er. = Erythema
Ed. = Edema

644

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APPENDIX I Riker Experiment No: 0386EB0578
PROTOCOL

TEST: Acute Primary Skin Irritation Test 645

SPONSOR: 3M RIKER Division

CONDUCTED BY: Pathology and Toxicology Department, Riker Laboratories, Inc. St. Paul, Minnesota

TEST ARTICLE: 1% Potassium Laurate (w/v solution in water & 5% Glycerin)

CONTROL ARTICLE: None

PROPOSED STARTING/COMPLETION DATE OF TEST: 10/86 - 12/86

TEST SYSTEM: New Zealand White Albino Rabbits (of either sex)

SOURCE: Hazleton Dutchland, Inc., Denver, Pa.

OBJECTIVE: To determine the primary irritation potential of the test article to the skin of 6 animals. Rabbits were selected as the test system due to their historical use, sensitivity to irritants, ease of handling and general availability.

METHOD: The animals will be housed in standard wire-mesh cages in temperature and humidity controlled rooms and food[a] and water offered *ad libitum.* Each animal will be assigned a numbered ear tag, which will correspond to a card affixed to the outside of cage. Prior to the application of the test article, the hair will be clipped from the back and flanks of each animal and 2 test sites selected lateral to the midline of the back approximately ten centimeters apart. 1 of the 2 sites will be abraded by making four epidermal incisions two perpendicular to the other two, while the other test site(s) will remain intact. The test article (0.5 ml) will be applied to 1 abraded and 1 intact site(s) on each animal and secured. The trunk of each animal will then be wrapped with impervous plastic sheeting which will occlude the test article during the 1 day exposure period. One hour and 2 days after removal of the test article during the 1 day exposure period. One hour and 2 days after removal of the test article, the intact and abraded test sites will be examined and scored separately for erythema and edema on a graded scale of 0 to 4. The average irritation produced will be evaluated by adding the mean scores for erythema and edema of the intact test sites one hour and 2 days post removal of the test article. Similarly, the mean scores for erythema and edema of the abraded test sites will be added. These two values will be totaled and divided by four to obtain the mean primary irritation index and then assigned a descriptive primary skin irritation rating as follows:

Mean Primary Irritation Score	Descriptive Rating
0	Non-irritating
0.1 - 0.5	Minimally Irritating
0.6 - 1.5	Slightly Irritating
1.6 - 3.0	Midly Irritating
3.1 - 5.0	Moderately Irritating
5.1 - 6.5	Severely Irritating
6.6 - 8.0	Extremely Irritating

The rating for a test article may be increased if the reaction caused is beyond erythema and edema and are deemed to be of importance in the interpretation of the results. All raw data generated by the study director and the final report will be stored in the Riker Laboratories' Archive, St. Paul, Minnesota.

[a] Purina Rabbit Chow, Ralston Purina Co., St. Louis, Missouri
[b] Draize: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965) Published by the Editorial Committee of the Association of Food and Drug Officials of the United States.

Sponsor 10/16/86 Date

Study Director 10/10/86 Date

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APPENDIX II

Principal Participating Personnel Involved in the Study

Name	Function
G. L. Harris, B.S.	Advanced Toxicologist Study Director
G. E. Hart	Master Laboratory Technician Acute Toxicology
L. O. Wiseth	Sr. Laboratory Technician Acute Toxicology
J. A. Eads	Jr. Laboratory Technician Acute Toxicology
J. L. Allen, Ph.D.	Research Toxicologist Acute Toxicology
G. C. Pecore	Supervisor Animal Laboratory

APPENDIX III

Composition Characteristics

This study is not regulated by the Good Laboratory Practice Act of 1978 and therefore information pertaining to composition characteristics is not applicable for inclusion in this study.

APPENDIX IV

Quality Assurance Statement

This study is not officially regulated by the Good Laboratory Practice Regulation of 1978, and therefore a statement signed and prepared by the Compliance Audit department is not applicable.

The standard operating procedures of this laboratory does adhere to the general principles of this regulation. The Compliance Audit department does inspect differenct significant phases for studies underway in the Acute Toxicology Laboratory on a recurring cycle, and the facilities are examined on a three month schedule. In addition a select number of Research & Development studies are routinely picked at random from the Archives by the Compliance Audit department for review.

Pathology and Toxicology
Riker Laboratories, Inc.

Building 270-3S-05, 3M Center
St Paul, Minnesota 55144-1000

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Primary Skin Irritation Test

with 50% S-26741 + 0.33% Sodium Lauryl Sulfate
in a Microporous Membrane ~0.5cm^2 Surface Area

in Albino Rabbits

Riker Experiment No: 0386EB0633

Conducted At: Pathology and Toxicology
Riker Laboratories, Inc.

Dates Conducted: October 28, 1986 to October 31, 1986

Conducted By: Gina L. Harris 11/20/86
G. L. Harris, B.S. Date
Advanced Toxicologist
Study Director and Acute Toxicity
Study Coordinator

Reviewed By: J. L. Allen 11-24-86
J. L. Allen, Ph.D. Date
Diplomate, A.B.T.
Research Toxicologist

dc: J. L. Allen
R. T. Catherall
C. F. Chesney
M. W. Downing
N. M. Marecki
M. J. Westfall
Tech. Doc. Cntr
Path/Tox Files

Summary

The results of the primary skin irritation test conducted from October 28, 1986 to October 31, 1986 at Riker Laboratories, Inc., St. Paul, Minnesota indicate that 50% S-26741 + 0.33% Sodium Lauryl Sulfate in a Microporous Membrane ~0.5 cm^2 Surface Area is slightly irritating (0.9/8.0) to the intact skin of female albino rabbits. Minimal erythema in 5/6 rabbits and slight to minimal edema in 2/6 rabbits were noted at the one hour evaluation following a one day occluded contact period. Minimal erythema and edema in 1/6 rabbits were noted at the final observation on day two.

Introduction

The objective of this study was to determine the primary skin irritation potential of 50% S-26741 + 0.33% Sodium Lauryl Sulfate in a Microporous Membrane ~0.5 cm^2 Surface Area to the skin of female albino rabbits. This study was conducted for research and development purposes and is, therefore, not regulated by the Food and Drug Administration's Good Laboratory Practice Regulation of 1978, although the standard operating procedures of this laboratory adhere to the general principles of this regulation. The raw data generated by the Study Director and the final report are stored in the conducting laboratory's archives.

Animals and Husbandry

Young New Zealand White Rabbits[a] were used in the evaluation of the primary skin irritating properties of the test article. The rabbits were individually housed[b] in stainless steel cages, and food[c] and water were available ad libitum. All rabbits were individually identified with ear tags and considered to be in good health at study initiation. The rabbits were housed in a temperature and humidity controlled room. Room lighting was on a 12/12 hour light/dark cycle that was automatically timed.

Method and Results

The test procedure was modeled after that of Draize et al[d]. One day prior to the application of the test article, the hair was clipped from the back and flanks of each rabbit and two test sites selected lateral to the midline of the back approximately ten centimeters apart.

The test article (~0.5 cm^2 surface area) was applied to one intact test site on each rabbit and immediately covered with two-inch square gauze patches. The patches, which were placed directly over the test sites, were secured with gauze wrap. The trunk of each animal was then wrapped with impervious plastic sheeting[e] which held the patches in position during the one day exposure period.

At the end of one day, the plastic wrappings, patches, and all residual test article were removed by washing with acetone. One hour and two days after removal of the test article, the intact test site was examined and scored separately for erythema and edema on a graded scale of 0 - 4.

The average irritation produced was evaluated by adding the mean scores for erythema and edema of the intact test sites one hour and two days post removal of the test article. These values were totaled and divided by two to obtain the mean primary irritation index.

[a] Hazleton Dutchland, Inc., Denver, PA
[b] Animals were housed in accordance with recommendations contained in DHEW Publication No. 78-23 (NIH): Revised 1985 "Guide For the Care and Use of Laboratory Animals.
[c] Purina Lab Rabbit Chow® and rabbits may be offered Alfalfa Cubes for additional roughage.
[d] Draize: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965).
[e] 10 x 12 x .002 Extra Clear polyethylene sleeves, PPC Industries, Inc., Wheeling, IL

The scoring criteria for erythema and edema are shown below.

Scoring Criteria for Skin Reactions

Reaction	Description	Score
Erythema	Barely perceptible (Edges of area not defined)	1
	Pale red in color and area definable	2
	Definite red in color and area well defined.	3
	Beet or crimson red in color	4
Edema	Barely perceptible (Edges of area not defined)	1
	Area definable but not raised more than 1 mm.	2
	Area well defined and raised approximately 1 mm.	3
	Area raised more than 1 mm.	4
	Maximum Primary Irritation Score =	8

The following grading system was used to arrive at a descriptive primary skin irritation rating:

Mean Primary Irritation Score (Range of Values)	Descriptive Rating
0	Non-Irritating
0.1 - 0.5	Minimally Irritating
0.6 - 1.5	Slightly Irritating
1.6 - 3.0	Mildly Irritating
3.1 - 5.0	Moderately Irritating
5.1 - 6.5	Severely Irritating
6.6 - 8.0	Extremely Irritating

The rating for a test article may be increased if the reactions caused are beyond simple erythema and edema, e.g. necrosis, escharosis, hemorrhage. The results are presented in Table 1. The protocol, principal personnel involved in the study, composition characteristics and Quality Assurance statement are contained in Appendices I - IV.

Table 1

Primary Skin Irritation Test - Albino Rabbits

with 50% S-26741 + 0.33% Sodium Lauryl Sulfate in a Microporous Membrane ~0.5 cm^2 Surface area

Animal Number	Irritation Scores for Intact Skin Sites after Removal: 1 Hour Er.	1 Hour Ed.	Day 2 Er.	Day 2 Ed.
6B1700	1	0	0	0
6B1703	0	0	0	0
6B1695	1	0	0	0
6B1698	1	2	0	0
6B1701	1	1	1	1
6B1704	1	0	0	0
Mean	0.8	0.5	0.2	0.2

Subtotal 1.7

Rating: Slightly irritating

Primary Irritation Index: 0.9/8.0

Key: Er. = Erythema
Ed. = Edema

APPENDIX I
PROTOCOL

Riker Experiment No: 0386EB0633

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654

TEST: Acute Primary Skin Irritation Test

SPONSOR: 3M Riker Division

CONDUCTED BY: Pathology and Toxicology Department, Riker Laboratories, Inc. St. Paul, Minnesota

Lot 326-38

TEST ARTICLE: 50% S-2741 + 0.33% sodium lauryl sulfate in a microporous membrane

CONTROL ARTICLE: NONE

PROPOSED STARTING/COMPLETION DATE OF TEST: 10/86 - 1/87

TEST SYSTEM: New Zealand White Albino Rabbits (of either sex)

SOURCE: Hazleton Dutchland, Inc., Denver, Pa.

OBJECTIVE. To determine the primary irritation potential of the test article to the skin of 6 animals. Rabbits were selected as the test system due to their historical use, sensitivity to irritants, ease of handling and general availability.

METHOD. The animals will be housed in standard wire-mesh cages in temperature and humidity controlled rooms and food[a] and water offered *ad libitum*. Each animal will be assigned a numbered ear tag. which will correspond to a card affixed to the outside of cage. Prior to the application of the test article, the hair will be clipped from the back and flanks of each animal and 1 test sites selected lateral to the midline of the back approximately ten centimeters apart. NONE of the TEST sites will be abraded by making four epidermal incisions two perpendicular to the other two, while the other test site(s) will remain intact. The test article (0.5 cm^2) will be applied to 0 abraded and 1 intact site(s) on each animal and secured. The trunk of each animal will then be wrapped with impervous plastic sheeting which will occlude the test article during the 1 day exposure period. One hour and 2 days after removal of the test article during the 1 day exposure period. One hour and 2 days after removal of the test article, the intact and abraded test sites will be examined and scored separately for erythema and edema on a graded scale of 0 to 4. The average irritation produced will be evaluated by adding the mean scores for erythema and edema of the intact test sites one hour and 2 days post removal of the test article. ~~Similarly, the mean scores for erythema and edema of the abraded test sites will be added~~. These two values will be totaled and divided by ~~four~~ TWO to obtain the mean primary irritation index and then assigned a descriptive primary skin irritation rating as follows:

Mean Primary Irritation Score	Descriptive Rating
0	Non-irritating
0.1 - 0.5	Minimally Irritating
0.6 - 1.5	Slightly Irritating
1.6 - 3.0	Midly Irritating
3.1 - 5.0	Moderately Irritating
5.1 - 6.5	Severely Irritating
6.6 - 8.0	Extremely Irritating

NOTE: INTACT SITE ONLY

The rating for a test article may be increased if the reaction caused is beyond erythema and edema and are deemed to be of importance in the interpretation of the results. All raw data generated by the study director and the final report will be stored in the Riker Laboratories' Archive, St. Paul, Minnesota.

[a] Purina Rabbit Chow, Ralston Purina Co., St. Louis, Missouri

[b] Draize: Apprasisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965) Published by the Editorial Committee of the Association of Food and Drug Officials of the United States.

Sponsor — Date 10/24/86

Study Director — Date 10/27/86

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APPENDIX II

Principal Participating Personnel Involved in the Study

Name	Function
G. L. Harris, B.S.	Advanced Toxicologist Study Director
G. E. Hart	Master Laboratory Technician Acute Toxicology
L. O. Wiseth	Sr. Laboratory Technician Acute Toxicology
J. A. Eads	Jr. Laboratory Technician Acute Toxicology
J. L. Allen, Ph.D.	Research Toxicologist Acute Toxicology
G. C. Pecore	Supervisor Animal Laboratory

APPENDIX III

Composition Characteristics

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APPENDIX IV

Quality Assurance Statement

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Pathology and Toxicology
Riker Laboratories, Inc.

Building 270-3S-05, 3M Center
St. Paul, Minnesota 55144-1000

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Primary Skin Irritation Test with 50% S-26741 + 0.35% Docusate Sodium in a Microporous Membrane ~0.5 cm^2 Surface Area in Albino Rabbits

Riker Experiment No: 0386EB0634

Conducted At: Pathology and Toxicology
Riker Laboratories, Inc.

Dates Conducted: October 28, 1986 to October 31, 1986

Conducted By: Gina L. Harris 11/20/86
G. L. Harris, B.S. Date
Advanced Toxicologist
Study Director and Acute Toxicity
Study Coordinator

Reviewed By: J. Allen 11-24-86
J. L. Allen, Ph.D. Date
Diplomate, A.B.T.
Research Toxicologist

dc: J. L. Allen
R. T. Catherall
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M. J. Westfall
Tech. Doc. Cntr.
Path/Tox Files

Summary

The results of the primary skin irritation test conducted from October 28, 1986 to October 31, 1986 at Riker Laboratories, Inc., St. Paul, Minnesota indicate that 50% S-26741 + 0.35% Docusate Sodium in a Microporous Membrane (~0.5 cm^2 Surface Area), is minimally irritating (0.3/8.0) to the intact skin of female albino rabbits. Slight erythema and edema in 1/6 rabbits were noted at the one hour evaluation following a one day occluded contact period. No dermal irritation was noted at the final observation on day two.

Introduction

The objective of this study was to determine the primary skin irritation potential of 50% S-26741 + 0.35% Docusate Sodium in a Microporous Membrane ~0.5 cm^2 Surface Area, to the skin of female albino rabbits. This study was conducted for research and development purposes and is, therefore, not regulated by the Food and Drug Administration's Good Laboratory Practice Regulation of 1978, although the standard operating procedures of this laboratory adhere to the general principles of this regulation. The raw data generated by the Study Director and the final report are stored in the conducting laboratory's archives.

Animals and Husbandry

Young New Zealand White Rabbits[a] were used in the evaluation of the primary skin irritating properties of the test article. The rabbits were individually housed[b] in stainless steel cages, and food[c] and water were available ad libitum. All rabbits were individually identified with ear tags and considered to be in good health at study initiation. The rabbits were housed in a temperature and humidity controlled room. Room lighting was on a 12/12 hour light/dark cycle that was automatically timed.

Method and Results

The test procedure was modeled after that of Draize et al[d]. One day prior to the application of the test article, the hair was clipped from the back and flanks of each rabbit and two test sites selected lateral to the midline of the back approximately ten centimeters apart.

The test article (~0.5 cm^2 surface area) was applied to one intact test site on each rabbit and immediately covered with two-inch square gauze patches. The patches, which were placed directly over the test sites, were secured with gauze wrap. The trunk of each animal was then wrapped with impervious plastic sheeting[e] which held the patches in position during the one day exposure period.

At the end of one day, the plastic wrappings, patches, and all residual test article were removed by washing with acetone. One hour and two days after removal of the test article, the intact test site was examined and scored separately for erythema and edema on a graded scale of 0 - 4.

The average irritation produced was evaluated by adding the mean scores for erythema and edema of the intact test sites one hour and two days post removal of the test article. These values were totaled and divided by two to obtain the mean primary irritation index.

[a] Hazleton Dutchland, Inc., Denver, PA
[b] Animals were housed in accordance with recommendations contained in DHEW Publication No. 78-23 (NIH): Revised 1985 "Guide For the Care and Use of Laboratory Animals.
[c] Purina Lab Rabbit Chow® and rabbits may be offered Alfalfa Cubes for additional roughage.
[d] Draize: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965).
[e] 10 x 12 x .002 Extra Clear polyethylene sleeves, PPC Industries, Inc., Wheeling, IL

The scoring criteria for erythema and edema are shown below.

Scoring Criteria for Skin Reactions

Reaction	Description	Score
Erythema	Barely perceptible (Edges of area not defined)	1
	Pale red in color and area definable	2
	Definite red in color and area well defined.	3
	Beet or crimson red in color	4
Edema	Barely perceptible (Edges of area not defined)	1
	Area definable but not raised more than 1 mm.	2
	Area well defined and raised approximately 1 mm.	3
	Area raised more than 1 mm.	4
	Maximum Primary Irritation Score =	8

The following grading system was used to arrive at a descriptive primary skin irritation rating:

Mean Primary Irritation Score (Range of Values)	Descriptive Rating
0	Non-Irritating
0.1 - 0.5	Minimally Irritating
0.6 - 1.5	Slightly Irritating
1.6 - 3.0	Mildly Irritating
3.1 - 5.0	Moderately Irritating
5.1 - 6.5	Severely Irritating
6.6 - 8.0	Extremely Irritating

The rating for a test article may be increased if the reactions caused are beyond simple erythema and edema, e.g. necrosis, escharosis, hemorrhage. The results are presented in Table 1. The protocol, principal personnel involved in the study, composition characteristics and Quality Assurance statement are contained in Appendices I - IV.

662

Table 1

Primary Skin Irritation Test - Albino Rabbits

with 50% S-26741 + 0.35% Docusate Sodium in a

Microporous Membrane ~0.5 cm^2 Surface Area

Animal Number	Irritation Scores for Intact Skin Sites after Removal: 1 Hour Er.	1 Hour Ed.	Day 2 Er.	Day 2 Ed.
6B1669	0	0	0	0
6B1672	0	0	0	0
6B1675	2	2	0	0
6B1678	0	0	0	0
6B1670	0	0	0	0
6B1673	0	0	0	0
Mean	0.3	0.3	0.0	0.0
Subtotal		0.6		

Rating: Minimally irritating

Primary Irritation Index: 0.3/8.0

Key: Er. = Erythema
Ed. = Edema

APPENDIX I
PROTOCOL

Riker Experiment No. 0386EB0634

663

TEST: Acute Primary Skin Irritation Test — GLP STUDY

SPONSOR: 3M Riker Division

CONDUCTED BY: Pathology and Toxicology Department, Riker Laboratories, Inc. St. Paul, Minnesota

TEST ARTICLE: 502 S-2741 + 0.35% Dowicil 200 in a microporous membrane, Lot 326-41

CONTROL ARTICLE: NONE

PROPOSED STARTING/COMPLETION DATE OF TEST: 10/86 - 1/87

TEST SYSTEM: New Zealand White Albino Rabbits (of either sex)

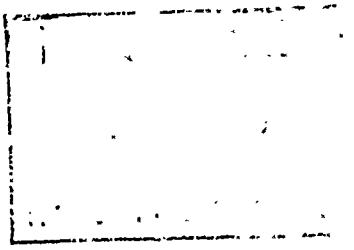
SOURCE: Hazleton Dutchland, Inc., Denver, Pa.

OBJECTIVE. To determine the primary irritation potential of the test article to the skin of 6 animals. Rabbits were selected as the test system due to their historical use, sensitivity to irritants, ease of handling and general availability.

METHOD. The animals will be housed in standard wire-mesh cages in temperature and humidity controlled rooms and food[a] and water offered *ad libitum*. Each animal will be assigned a numbered ear tag, which will correspond to a card affixed to the outside of cage. Prior to the application of the test article, the hair will be clipped from the back and flanks of each animal and 1 test sites selected lateral to the midline of the back approximately ten centimeters apart. NONE of the TEST sites will be abraded by making four epidermal incisions two perpendicular to the other two, while the other test site(s) will remain intact. The test article (0.5 cm.^{2}, will be applied to 0 abraded and 1 intact site(s) on each animal and secured. The trunk of each animal will then be wrapped with impervous plastic sheeting which will occlude the test article during the 1 day exposure period. One hour and 2 days after removal of the test article during the 1 day exposure period. One hour and 2 days after removal of the test article, the intact and abraded test sites will be examined and scored separately for erythema and edema on a graded scale of 0 to 4. The average irritation produced will be evaluated by adding the mean scores for erythema and edema of the intact test sites one hour and 2 days post removal of the test article. ~~Similarly, the mean scores for erythema and edema of the abraded test sites will be added.~~ These two values will be totaled and divided by ~~four~~ two to obtain the mean primary irritation index and then assigned a descriptive primary skin irritation rating as follows:

NOTE: Intact site only

Mean Primary Irritation Score	Descriptive Rating
0	Non-irritating
0.1 - 0.5	Minimally Irritating
0.6 - 1.5	Slightly Irritating
1.6 - 3.0	Midly Irritating
3.1 - 5.0	Moderately Irritating
5.1 - 6.5	Severely Irritating
6.6 - 8.0	Extremely Irritating



The rating for a test article may be increased if the reaction caused is beyond erythema and edema and are deemed to be of importance in the interpretation of the results. All raw data generated by the study director and the final report will be stored in the Riker Laboratories' Archive, St. Paul, Minnesota.

[a] Purina Rabbit Chow, Ralston Purina Co., St. Louis, Missouri

[b] Draize: Apprasisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965) Published by the Editorial Committee of the Association of Food and Drug Officials of the United States.

Sponsor — 10/24/86 Date

Study Director — 10/27/86 Date

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APPENDIX II

Principal Participating Personnel Involved in the Study

Name	Function
G. L. Harris, B.S.	Advanced Toxicologist Study Director
G. E. Hart	Master Laboratory Technician Acute Toxicology
L. O. Wiseth	Sr. Laboratory Technician Acute Toxicology
J. A. Eads	Jr. Laboratory Technician Acute Toxicology
J. L. Allen, Ph.D.	Research Toxicologist Acute Toxicology
G. C. Pecore	Supervisor Animal Laboratory

APPENDIX III

Composition Characteristics

This study is not regulated by the Good Laboratory Practice Act of 1978 and therefore information pertaining to composition characteristics is not applicable for inclusion in this study.

APPENDIX IV

Quality Assurance Statement

This study is not officially regulated by the Good Laboratory Practice Regulation of 1978, and therefore a statement signed and prepared by the Compliance Audit department is not applicable.

The standard operating procedures of this laboratory does adhere to the general principles of this regulation. The Compliance Audit department does inspect differenct significant phases for studies underway in the Acute Toxicology Laboratory on a recurring cycle, and the facilities are examined on a three month schedule. In addition a select number of Research & Development studies are routinely picked at random from the Archives by the Compliance Audit department for review.

Pathology and Toxicology
Riker Laboratories, Inc.

Building 270-3S-05, 3M Center
St. Paul, Minnesota 55144-1000

COMPANY CONFIDENTIAL

3M

Primary Skin Irritation Test

with 50% S-26741 + 0.35% Docusate Sodium in a Gel

~0.5 cm^2 Surface Area

in Albino Rabbits

Riker Experiment No: 0386EB0635

Conducted At: Pathology and Toxicology
Riker Laboratories, Inc.

Dates Conducted: October 28, 1986 to October 31, 1986

Conducted By: Gene L. Harris 11/26/86
G. L. Harris, B.S. Date
Advanced Toxicologist
Study Director and Acute Toxicity
Study Coordinator

Reviewed By: J Allen 11-25-86
J. L. Allen, Ph.D. Date
Diplomate, A.B.T.
Research Toxicologist

dc: J. L. Allen
R. T. Catherall
C. F. Chesney
M. W. Downing
N. M. Marecki
M. J. Westfall
Tech. Doc. Cntr.
Path/Tox Files

Summary

The results of the primary skin irritation test conducted from October 28, 1986 to October 31, 1986 at Riker Laboratories, Inc., St. Paul, Minnesota indicate that 50% S-26741 + 0.35% Docusate Sodium in a Gel (~0.5 cm^2 surface area), is slightly irritating (1.1/8.0) to the intact skin of female albino rabbits. Minimal to slight erythema in 5/6 rabbits and slight edema in 2/6 rabbits were noted at the one hour evaluation following a one day occluded contact period. Minimal erythema in 1/6 rabbits was noted at the final observation on day two. The pharmacotoxic signs observed in 1/6 rabbits six hours after dose administration were diarrhea, tremors and salivation.

Introduction

The objective of this study was to determine the primary skin irritation potential of 50% S-26741 + 0.35% Docusate Sodium in a Gel (~0.5 cm^2 surface area), to the skin of female albino rabbits. This study was conducted for research and development purposes and is, therefore, not regulated by the Food and Drug Administration's Good Laboratory Practice Regulation of 1978, although the standard operating procedures of this laboratory adhere to the general principles of this regulation. The raw data generated by the Study Director and the final report are stored in the conducting laboratory's archives.

Animals and Husbandry

Young New Zealand White Rabbits[a] were used in the evaluation of the primary skin irritating properties of the test article. The rabbits were individually housed[b] in stainless steel cages, and food[c] and water were available ad libitum. All rabbits were individually identified with ear tags and considered to be in good health at study initiation. The rabbits were housed in a temperature and humidity controlled room. Room lighting was on a 12/12 hour light/dark cycle that was automatically timed.

Method and Results

The test procedure was modeled after that of Draize et al[d]. One day prior to the application of the test article, the hair was clipped from the back and flanks of each rabbit and two test sites selected lateral to the midline of the back approximately ten centimeters apart.

The test article (~0.5 cm^2 surface area) was applied to one intact test site on each rabbit and immediately covered with two-inch square gauze patches. The patches, which were placed directly over the test sites, were secured with gauze wrap. The trunk of each animal was then wrapped with impervious plastic sheeting[e] which held the patches in position during the one day exposure period.

At the end of one day, the plastic wrappings, patches, and all residual test article were removed by washing with acetone. One hour and two days after removal of the test article, the intact test site was examined and scored separately for erythema and edema on a graded scale of 0 - 4.

The average irritation produced was evaluated by adding the mean scores for erythema and edema of the intact test sites one hour and two days post removal of the test article. These values were totaled and divided by two to obtain the mean primary irritation index.

[a] Hazleton Dutchland, Inc., Denver, PA
[b] Animals were housed in accordance with recommendations contained in DHEW Publication No. 78-23 (NIH): Revised 1985 "Guide For the Care and Use of Laboratory Animals.
[c] Purina Lab Rabbit Chow® and rabbits may be offered Alfalfa Cubes for additional roughage.
[d] Draize: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965).
[e] 10 x 12 x .002 Extra Clear polyethylene sleeves, PPC Industries, Inc., Wheeling, IL

The scoring criteria for erythema and edema are shown below:

Scoring Criteria for Skin Reactions

Reaction	Description	Score
Erythema	Barely perceptible (Edges of area not defined)	1
	Pale red in color and area definable	2
	Definite red in color and area well defined.	3
	Beet or crimson red in color	4
Edema	Barely perceptible (Edges of area not defined)	1
	Area definable but not raised more than 1 mm.	2
	Area well defined and raised approximately 1 mm.	3
	Area raised more than 1 mm.	4
	Maximum Primary Irritation Score =	8

The following grading system was used to arrive at a descriptive primary skin irritation rating:

Mean Primary Irritation Score (Range of Values)	Descriptive Rating
0	Non-Irritating
0.1 - 0.5	Minimally Irritating
0.6 - 1.5	Slightly Irritating
1.6 - 3.0	Mildly Irritating
3.1 - 5.0	Moderately Irritating
5.1 - 6.5	Severely Irritating
6.6 - 8.0	Extremely Irritating

The rating for a test article may be increased if the reactions caused are beyond simple erythema and edema, e.g. necrosis, escharosis, hemorrhage. The results are presented in Table 1. The protocol, principal personnel involved in the study, composition characteristics and Quality Assurance statement are contained in Appendices I - IV.

Table 1

Primary Skin Irritation Test - Albino Rabbits

with 50% S-26741 + 0.35% Docusate Sodium in a Gel

~0.5 cm^2 Surface Area

Animal Number	Irritation Scores for Intact Skin Sites after Removal: 1 Hour Er.	1 Hour Ed.	Day 2 Er.	Day 2 Ed.
6B1705*	1	0	0	0
6B1708	2	0	0	0
6B1711	1	2	0	0
6B1714	0	0	0	0
6B1706	2	2	1	0
6B1709	1	0	0	0
Mean	1.2	0.7	0.2	0.0

Subtotal 2.1

Rating: Slightly irritating

Primary Irritation Index: 1.1/8.0

Key: Er. = Erythema
Ed. = Edema
* = Diarrhea, tremors, salivation 4 hours post dose

APPENDIX I Riker Experiment No: 0386EB0635
PROTOCOL

TEST: Acute Primary Skin Irritation Test NON-CU ... 672

SPONSOR: 3M Riker Division

CONDUCTED BY: Pathology and Toxicology Department, Riker Laboratories, Inc. St. Paul, Minnesota

TEST ARTICLE: 50% S-2674 + 0.35% Docusate Sodium in a Gel, Lot 325-12B

CONTROL ARTICLE: NONE

PROPOSED STARTING/COMPLETION DATE OF TEST: 10/86 - 1/87

TEST SYSTEM: New Zealand White Albino Rabbits (of either sex)

SOURCE: Hazleton Dutchland, Inc., Denver, Pa.

OBJECTIVE. To determine the primary irritation potential of the test article to the skin of 6 animals. Rabbits were selected as the test system due to their historical use, sensitivity to irritants, ease of handling and general availability.

METHOD. The animals will be housed in standard wire-mesh cages in temperature and humidity controlled rooms and food[a] and water offered *ad libitum.* Each animal will be assigned a numbered ear tag, which will correspond to a card affixed to the outside of cage. Prior to the application of the test article, the hair will be clipped from the back and flanks of each animal and 1 test sites selected lateral to the midline of the back approximately ten centimeters apart. NONE of the Test sites will be abraded by making four epidermal incisions two perpendicular to the other two, while the other test site(s) will remain intact. The test article (_____) will be applied to NO abraded and 1 intact site(s) on each animal and secured. The trunk of each animal will then be wrapped with impervous plastic sheeting which will occlude the test article during the 1 day exposure period. One hour and 2 days after removal of the test article during the 1 day exposure period. One hour and 2 days after removal of the test article, the intact ~~and abraded~~ test sites will be examined and scored separately for erythema and edema on a graded scale of 0 to 4. The average irritation produced will be evaluated by adding the mean scores for erythema and edema of the intact test sites one hour and 2 days post removal of the test article. ~~Similarly, the mean scores for erythema and edema of the abraded test sites will be added.~~ These two values will be totaled and divided by ~~four~~ two to obtain the mean primary irritation index and then assigned a descriptive primary skin irritation rating as follows:

Mean Primary Irritation Score	Descriptive Rating
0	Non-irritating
0.1 - 0.5	Minimally Irritating
0.6 - 1.5	Slightly Irritating
1.6 - 3.0	Midly Irritating
3.1 - 5.0	Moderately Irritating
5.1 - 6.5	Severely Irritating
6.6 - 8.0	Extremely Irritating

NOTE: THE TEST MATERIAL WILL BE ADMINISTERED TO ONLY ONE INTACT SITE PER RABBIT.

The rating for a test article may be increased if the reaction caused is beyond erythema and edema and are deemed to be of importance in the interpretation of the results. All raw data generated by the study director and the final report will be stored in the Riker Laboratories' Archive, St. Paul, Minnesota.

[a] Purina Rabbit Chow, Ralston Purina Co., St. Louis, Missouri

[b] Draize: Apprasisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965) Published by the Editorial Committee of the Association of Food and Drug Officials of the United States.

Nelda K. Garcia 10/24/86
Sponsor Date

Glen L Harris 10/27/86
Study Director Date

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APPENDIX II

Principal Participating Personnel Involved in the Study

Name	Function
G. L. Harris, B.S.	Advanced Toxicologist Study Director
G. E. Hart	Master Laboratory Technician Acute Toxicology
L. O. Wiseth	Sr. Laboratory Technician Acute Toxicology
J. A. Eads	Jr. Laboratory Technician Acute Toxicology
J. L. Alien, Ph.D.	Research Toxicologist Acute Toxicology
G. C. Pecore	Supervisor Animal Laboratory

APPENDIX III

Composition Characteristics

This study is not regulated by the Good Laboratory Practice Act of 1978 and therefore information pertaining to composition characteristics is not applicable for inclusion in this study.

675

APPENDIX IV

Quality Assurance Statement

This study is not officially regulated by the Good Laboratory Practice Regulation of 1978, and therefore a statement signed and prepared by the Compliance Audit department is not applicable.

The standard operating procedures of this laboratory does adhere to the general principles of this regulation. The Compliance Audit department does inspect differenct significant phases for studies underway in the Acute Toxicology Laboratory on a recurring cycle, and the facilities are examined on a three month schedule. In addition a select number of Research & Development studies are routinely picked at random from the Archives by the Compliance Audit department for review.

Pathology and Toxicology
Riker Laboratories, Inc.

Building 270-3S-05, 3M Center
St. Paul, Minnesota 55144-1000

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Primary Skin Irritation Test

with 50% S-26741 + 0.33% Sodium Lauryl Sulfate in a Gel

~0.5 cm^2 Surface Area

in Albino Rabbits

Riker Experiment No: 0386EB0636

Conducted At: Pathology and Toxicology
Riker Laboratories, Inc.

Dates Conducted: October 18, 1986 to October 31, 1986

Conducted By: G. L. Harris 11/24/86

G. L. Harris, B.S. Date
Advanced Toxicologist
Study Director and Acute Toxicity
Study Coordinator

Reviewed By: J. Allen 11-25-86

J. L. Allen, Ph.D. Date
Diplomate, A.B.T.
Research Toxicologist

dc: J. L. Allen
R. T. Catherall
C. F. Chesney
M. W. Downing
N. M. Marecki
M. J. Westfall
Tech. Doc. Cntr
Path/Tox Files

Summary

The results of the primary skin irritation test conducted from October 18, 1986 to October 31, 1986 at Riker Laboratories, Inc., St. Paul, Minnesota indicate that 50% S-26741 + 0.33% Sodium Lauryl Sulfate in a Gel ~0.5 cm^2 Surface Area, is slightly irritating (0.8/8.0) to the skin of female albino rabbits. Minimal erythema in 5/6 rabbits and slight edema in 1/6 rabbits were noted at the one hour evaluation following a one day occluded contact period. Minimal erythema in 3/6 rabbits was noted at the final observation on day two. The pharmacotoxic signs noted during this study were tremors in 1/6 rabbits at two hours, and tremors and diarrhea in 1/6 rabbits at four hours post dose.

Introduction

The objective of this study was to determine the primary skin irritation potential of 50% S-26741 + 0.33% Sodium Lauryl Sulfate in a Gel ~0.5 cm^2 Surface Area, to the skin of female albino rabbits. This study was conducted for research and development purposes and is, therefore, not regulated by the Food and Drug Administration's Good Laboratory Practice Regulation of 1978, although the standard operating procedures of this laboratory adhere to the general principles of this regulation. The raw data generated by the Study Director and the final report are stored in the conducting laboratory's archives.

Animals and Husbandry

Young New Zealand White Rabbits[a] were used in the evaluation of the primary skin irritating properties of the test article. The rabbits were individually housed[b] in stainless steel cages, and food[c] and water were available ad libitum. All rabbits were individually identified with ear tags and considered to be in good health at study initiation. The rabbits were housed in a temperature and humidity controlled room. Room lighting was on a 12/12 hour light/dark cycle that was automatically timed.

Method and Results

The test procedure was modeled after that of Draize et al[d]. One day prior to the application of the test article, the hair was clipped from the back and flanks of each rabbit and two test sites selected lateral to the midline of the back approximately ten centimeters apart.

The test article (~0.5 cm^2 surface area) was applied to one intact test site on each rabbit and immediately covered with two-inch square gauze patches. The patches, which were placed directly over the test sites, were secured with gauze wrap. The trunk of each animal was then wrapped with impervious plastic sheeting[e] which held the patches in position during the one day exposure period.

At the end of one day, the plastic wrappings, patches, and all residual test article were removed by washing with acetone. One hour and two days after removal of the test article, the intact test site was examined and scored separately for erythema and edema on a graded scale of 0 - 4.

The average irritation produced was evaluated by adding the mean scores for erythema and edema of the intact test sites one hour and two days post removal of the test article. These values were totaled and divided by two to obtain the mean primary irritation index.

[a] Hazleton Dutchland, Inc., Denver, PA
[b] Animals were housed in accordance with recommendations contained in DHEW Publication No. 78-23 (NIH): Revised 1985 "Guide For the Care and Use of Laboratory Animals.
[c] Purina Lab Rabbit Chow® and rabbits may be offered Alfalfa Cubes for additional roughage.
[d] Draize: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965).
[e] 10 x 12 x .002 Extra Clear polyethylene sleeves, PPC Industries, Inc., Wheeling, IL

The scoring criteria for erythema and edema are shown below.

Scoring Criteria for Skin Reactions

Reaction	Description	Score
Erythema	Barely perceptible (Edges of area not defined)	1
	Pale red in color and area definable	2
	Definite red in color and area well defined.	3
	Beet or crimson red in color	4
Edema	Barely perceptible (Edges of area not defined)	1
	Area definable but not raised more than 1 mm.	2
	Area well defined and raised approximately 1 mm.	3
	Area raised more than 1 mm.	4
	Maximum Primary Irritation Score =	8

The following grading system was used to arrive at a descriptive primary skin irritation rating:

Mean Primary Irritation Score (Range of Values)	Descriptive Rating
0	Non-Irritating
0.1 - 0.5	Minimally Irritating
0.6 - 1.5	Slightly Irritating
1.6 - 3.0	Mildly Irritating
3.1 - 5.0	Moderately Irritating
5.1 - 6.5	Severely Irritating
6.6 - 8.0	Extremely Irritating

The rating for a test article may be increased if the reactions caused are beyond simple erythema and edema, e.g. necrosis, escharosis, hemorrhage. The results are presented in Table 1. The protocol, principal personnel involved in the study, composition characteristics and Quality Assurance statement are contained in Appendices I - IV.

680

Table 1

Primary Skin Irritation Test - Albino Rabbits

with 50% S-26741 + 0.33% Sodium Lauryl Sulfate in a Gel

~0.5 cm^2 Surface Area

Animal Number	Irritation Scores for Intact Skin Sites after Removal: 1 Hour Er.	1 Hour Ed.	Day 2 Er.	Day 2 Ed.
6B1676*	1	0	1	0
6B1679	1	0	1	0
6B1671	1	2	1	0
6B1674	1	0	0	0
6B1677	0	0	0	0
6B1680**	1	0	0	0
Mean	0.8	0.3	0.5	0.0
Subtotal			1.6	

Rating: Slightly irritating

Primary Irritation Index: 0.8/8.0

Key: Er. = Erythema
Ed. = Edema
* = Tremors 2 hours post dose
** = Tremors, diarrhea 4 hours post dose

APPENDIX I
PROTOCOL

Riker Experiment No: 0386EB0636

TEST: Acute Primary Skin Irritation Test NON-GLP STUDY 681 5.

SPONSOR: 3M RIKER Division

CONDUCTED BY: Pathology and Toxicology Department, Riker Laboratories, Inc. St. Paul, Minnesota

TEST ARTICLE: 50% S-2674 + 0.33% sodium lauryl sulfate in 9 Gel, Lot 325-12A

CONTROL ARTICLE: NONE

PROPOSED STARTING/COMPLETION DATE OF TEST: 10/86 - 1/87

TEST SYSTEM: New Zealand White Albino Rabbits (of either sex)

SOURCE: Hazleton Dutchland. Inc., Denver, Pa.

OBJECTIVE. To determine the primary irritation potential of the test article to the skin of 6 animals. Rabbits were selected as the test system due to their historical use, sensitivity to irritants, ease of handling and general availability.

METHOD: The animals will be housed in standard wire-mesh cages in temperature and humidity controlled rooms and food[a] and water offered *ad libitum*. Each animal will be assigned a numbered ear tag, which will correspond to a card affixed to the outside of cage. Prior to the application of the test article, the hair will be clipped from the back and flanks of each animal and 1 test sites selected lateral to the midline of the back approximately ten centimeters apart. None of the Test sites will be abraded by making four epidermal incisions two perpendicular to the other two, while the other test site(s) will remain intact. The test article (0.5 cm^2) will be applied to 0 abraded and 1 intact site(s) on each animal and secured. The trunk of each animal will then be wrapped with impervous plastic sheeting which will occlude the test article during the 1 day exposure period. One hour and 2 days after removal of the test article during the 1 day exposure period. One hour and 2 days after removal of the test article, the intact and abraded test sites will be examined and scored separately for erythema and edema on a graded scale of 0 to 4. The average irritation produced will be evaluated by adding the mean scores for erythema and edema of the intact test sites one hour and 2 days post removal of the test article. ~~Similarly, the mean scores for erythema and edema of the abraded test sites will be added~~. These two values will be totaled and divided by ~~four~~ two to obtain the mean primary irritation index and then assigned a descriptive primary skin irritation rating as follows:

NOTE: Intact Site Only

Mean Primary Irritation Score	Descriptive Rating
0	Non-irritating
0.1 - 0.5	Minimally Irritating
0.6 - 1.5	Slightly Irritating
1.6 - 3.0	Midly Irritating
3.1 - 5.0	Moderately Irritating
5.1 - 6.5	Severely Irritating
6.6 - 8.0	Extremely Irritating

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The rating for a test article may be increased if the reaction caused is beyond erythema and edema and are deemed to be of importance in the interpretation of the results. All raw data generated by the study director and the final report will be stored in the Riker Laboratories' Archive, St. Paul, Minnesota.

[a] Purina Rabbit Chow, Ralston Purina Co., St. Louis, Missouri

[b] Draize: Apprasisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965) Published by the Editorial Committee of the Association of Food and Drug Officials of the United States.

Nelda K. Garrecht 10/24/86
Sponsor Date

Gene L. Harris 10/27/86
Study Director Date

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682

APPENDIX II

Principal Participating Personnel Involved in the Study

Name	Function
G. L. Harris, B.S.	Advanced Toxicologist Study Director
G. E. Hart	Master Laboratory Technician Acute Toxicology
L. O. Wiseth	Sr. Laboratory Technician Acute Toxicology
J. A. Eads	Jr. Laboratory Technician Acute Toxicology
J. L. Allen, Ph.D.	Research Toxicologist Acute Toxicology
G. C. Pecore	Supervisor Animal Laboratory

APPENDIX III

Composition Characteristics

This study is not regulated by the Good Laboratory Practice Act of 1978 and therefore information pertaining to composition characteristics is not applicable for inclusion in this study.

APPENDIX IV

Quality Assurance Statement

This study is not officially regulated by the Good Laboratory Practice Regulation of 1978, and therefore a statement signed and prepared by the Compliance Audit department is not applicable.

The standard operating procedures of this laboratory does adhere to the general principles of this regulation. The Compliance Audit department does inspect differenct significant phases for studies underway in the Acute Toxicology Laboratory on a recurring cycle, and the facilities are examined on a three month schedule. In addition a select number of Research & Development studies are routinely picked at random from the Archives by the Compliance Audit department for review.

Pathology and Toxicology
Riker Laboratories, Inc.

Building 270-3S-05, 3M Center
St. Paul, Minnesota 55144-1000

COMPANY CONFIDENTIAL

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Primary Skin Irritation Test

with 50% S-26741 + 0.35% Docusate Sodium in Solution with Water and 5% Glycerin

in Albino Rabbits

Riker Experiment No: 0386EB0637

Conducted At: Pathology and Toxicology
Riker Laboratories, Inc.

Dates Conducted: October 28, 1986 to October 31, 1986

Conducted By: Gene F. Harris 11/26/86
G. L. Harris, B.S. Date
Advanced Toxicologist
Study Director and Acute Toxicity
Study Coordinator

Reviewed By: J L Allen 11-24-86
J. L. Allen, Ph.D. Date
Diplomate, A.B.T.
Research Toxicologist

dc: J. L. Allen
R. T. Catherall
C. F. Chesney
M. W. Downing
N. M. Marecki
M. J. Westfall
Tech. Doc. Cntr.
Path/Tox Files

Summary

The results of the primary skin irritation test conducted from October 28, 1986 to October 31, 1986 at Riker Laboratories, Inc., St. Paul, Minnesota indicate that 50% S-26741 + 0.35% Docusate Sodium in Solution with Water and 5% Glycerin, is non-irritating (0.0/8.0) to the intact skin of female albino rabbits. Neither erythema nor edema were noted at any time during the study.

Introduction

The objective of this study was to determine the primary skin irritation potential of 50% S-26741 + 0.35% Docusate Sodium in Solution with Water and 5% Glycerin, to the skin of female albino rabbits. This study was conducted for research and development purposes and is, therefore, not regulated by the Food and Drug Administration's Good Laboratory Practice Regulation of 1978, although the standard operating procedures of this laboratory adhere to the general principles of this regulation. The raw data generated by the Study Director and the final report are stored in the conducting laboratory's archives.

Animals and Husbandry

Young New Zealand White Rabbits[a] were used in the evaluation of the primary skin irritating properties of the test article. The rabbits were individually housed[b] in stainless steel cages, and food[c] and water were available ad libitum. All rabbits were individually identified with ear tags and considered to be in good health at study initiation. The rabbits were housed in a temperature and humidity controlled room. Room lighting was on a 12/12 hour light/dark cycle that was automatically timed.

Method and Results

The test procedure was modeled after that of Draize et al[d]. One day prior to the application of the test article, the hair was clipped from the back and flanks of each rabbit and two test sites selected lateral to the midline of the back approximately ten centimeters apart.

The test article (0.02 ml) was applied to one intact test site on each rabbit and immediately covered with two-inch square gauze patches. The patches, which were placed directly over the test sites, were secured with gauze wrap. The trunk of each animal was then wrapped with impervious plastic sheeting[e] which held the patches in position during the one day exposure period.

At the end of one day, the plastic wrappings, patches, and all residual test article were removed by washing with water. One hour and two days after removal of the test article, the intact test site was examined and scored separately for erythema and edema on a graded scale of 0 - 4.

The average irritation produced was evaluated by adding the mean scores for erythema and edema of the intact test sites one hour and two days post removal of the test article. These values were totaled and divided by two to obtain the mean primary irritation index.

[a] Hazleton Dutchland, Inc., Denver, PA
[b] Animals were housed in accordance with recommendations contained in DHEW Publication No. 78-23 (NIH): Revised 1985 "Guide For the Care and Use of Laboratory Animals.
[c] Purina Lab Rabbit Chow® and rabbits may be offered Alfalfa Cubes for additional roughage.
[d] Draize: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965).
[e] 10 x 12 x .002 Extra Clear polyethylene sleeves, PPC Industries, Inc., Wheeling, IL

The scoring criteria for erythema and edema are shown below.

Scoring Criteria for Skin Reactions

Reaction	Description	Score
Erythema	Barely perceptible (Edges of area not defined)	1
	Pale red in color and area definable	2
	Definite red in color and area well defined.	3
	Beet or crimson red in color	4
Edema	Barely perceptible (Edges of area not defined)	1
	Area definable but not raised more than 1 mm.	2
	Area well defined and raised approximately 1 mm.	3
	Area raised more than 1 mm.	4
	Maximum Primary Irritation Score =	8

The following grading system was used to arrive at a descriptive primary skin irritation rating:

Mean Primary Irritation Score (Range of Values)	Descriptive Rating
0	Non-Irritating
0.1 - 0.5	Minimally Irritating
0.6 - 1.5	Slightly Irritating
1.6 - 3.0	Mildly Irritating
3.1 - 5.0	Moderately Irritating
5.1 - 6.5	Severely Irritating
6.6 - 8.0	Extremely Irritating

The rating for a test article may be increased if the reactions caused are beyond simple erythema and edema, e.g. necrosis, escharosis, hemorrhage. The results are presented in Table 1. The protocol, principal personnel involved in the study, composition characteristics and Quality Assurance statement are contained in Appendices I - IV.

Table 1

Primary Skin Irritation Test - Albino Rabbits

with 50% S-26741 + 0.35% Docusate Sodium in Solution with Water and 5% Glycerin

Animal Number	Irritation Scores for Intact Skin Sites after Removal: 1 Hour Er.	1 Hour Ed.	Day 2 Er.	Day 2 Ed.
6B1693	0	0	0	0
6B1696	0	0	0	0
6B1699	0	0	0	0
6B1702	0	0	0	0
6B1694	0	0	0	0
6B1697	0	0	0	0
Mean	0.0	0.0	0.0	0.0

Subtotal 0.0

Rating: Non-irritating

Primary Irritation Index: 0.0 /8.0

Key: Er. = Erythema
Ed. = Edema

APPENDIX I
PROTOCOL

Riker Experiment No: 0386EB0637

5.

690

TEST: Acute Primary Skin Irritation Test

SPONSOR: 3M Riker Division

CONDUCTED BY: Pathology and Toxicology Department, Riker Laboratories, Inc. St. Paul, Minnesota

TEST ARTICLE: 50% S-2674I + 0.35% Docusate Sodium in solution with water & 5% Glycerin

CONTROL ARTICLE: NONE

PROPOSED STARTING/COMPLETION DATE OF TEST: 10/86 - 1/87

TEST SYSTEM: New Zealand White Albino Rabbits (of either sex)

SOURCE: Hazleton Dutchland, Inc., Denver, Pa.

OBJECTIVE. To determine the primary irritation potential of the test article to the skin of 6 animals. Rabbits were selected as the test system due to their historical use, sensitivity to irritants, ease of handling and general availability.

METHOD. The animals will be housed in standard wire-mesh cages in temperature and humidity controlled rooms and food[a] and water offered *ad libitum*. Each animal will be assigned a numbered ear tag, which will correspond to a card affixed to the outside of cage. Prior to the application of the test article, the hair will be clipped from the back and flanks of each animal and 1 test sites selected lateral to the midline of the back approximately ten centimeters apart. Nine of the TEST sites will be abraded by making four epidermal incisions two perpendicular to the other two, while the other test site(s) will remain intact. The test article (0.02 ml) will be applied to 0 abraded and 1 intact site(s) on each animal and secured. The trunk of each animal will then be wrapped with impervous plastic sheeting which will occlude the test article during the 1 day exposure period. One hour and 2 days after removal of the test article during the 1 day exposure period. One hour and 2 days after removal of the test article, the intact and abraded test sites will be examined and scored separately for erythema and edema on a graded scale of 0 to 4. The average irritation produced will be evaluated by adding the mean scores for erythema and edema of the intact test sites one hour and 2 days post removal of the test article. ~~Similarly, the mean scores for erythema and edema of the abraded test sites will be added~~. These two values will be totaled and divided by ~~four~~ two to obtain the mean primary irritation index and then assigned a descriptive primary skin irritation rating as follows:

NOTE: INTACT site ONLY

Mean Primary Irritation Score	Descriptive Rating
0	Non-irritating
0.1 - 0.5	Minimally Irritating
0.6 - 1.5	Slightly Irritating
1.6 - 3.0	Midly Irritating
3.1 - 5.0	Moderately Irritating
5.1 - 6.5	Severely Irritating
6.6 - 8.0	Extremely Irritating

The rating for a test article may be increased if the reaction caused is beyond erythema and edema and are deemed to be of importance in the interpretation of the results. All raw data generated by the study director and the final report will be stored in the Riker Laboratories' Archive, St. Paul, Minnesota.

[a] Purina Rabbit Chow, Ralston Purina Co., St. Louis, Missouri

[b] Draize: Apprasisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965) Published by the Editorial Committee of the Association of Food and Drug Officials of the United States.

Sponsor 10/28/86 Date

Study Director 10/27/86 Date

Form 19171-12-F PWO

APPENDIX II

Principal Participating Personnel Involved in the Study

Name	Function
G. L. Harris, B.S.	Advanced Toxicologist Study Director
G. E. Hart	Master Laboratory Technician Acute Toxicology
L. O. Wiseth	Sr. Laboratory Technician Acute Toxicology
J. A. Eads	Jr. Laboratory Technician Acute Toxicology
J. L. Allen, Ph.D.	Research Toxicologist Acute Toxicology
G. C. Pecore	Supervisor Animal Laboratory

APPENDIX III

Composition Characteristics

This study is not regulated by the Good Laboratory Practice Act of 1978 and therefore information pertaining to composition characteristics is not applicable for inclusion in this study.

APPENDIX IV

Quality Assurance Statement

This study is not officially regulated by the Good Laboratory Practice Regulation of 1978, and therefore a statement signed and prepared by the Compliance Audit department is not applicable.

The standard operating procedures of this laboratory does adhere to the general principles of this regulation. The Compliance Audit department does inspect differenct significant phases for studies underway in the Acute Toxicology Laboratory on a recurring cycle, and the facilities are examined on a three month schedule. In addition a select number of Research & Development studies are routinely picked at random from the Archives by the Compliance Audit department for review.

Pathology and Toxicology
Riker Laboratories, Inc.

Building 270-3S-05, 3M Center
St. Paul, Minnesota 55144-1000

COMPANY CONFIDENTIAL

3M

Primary Skin Irritation Test

with 50% S-26741 + 0.33% Sodium Lauryl Sulfate in Solution with Water and 5% Glycerin

in Albino Rabbits

Riker Experiment No: 0386EB0638

Conducted At: Pathology and Toxicology
Riker Laboratories, Inc.

Dates Conducted: October 28, 1986 to October 31, 1986

Conducted By: Gene L. Harris 11/20/86
G. L. Harris, B.S. Date
Advanced Toxicologist
Study Director and Acute Toxicity
Study Coordinator

Reviewed By: J Allen 11-24-86
J. L. Allen, Ph.D. Date
Diplomate, A.B.T.
Research Toxicologist

dc: J. L. Allen
R. T. Catherall
C. F. Chesney
M. W. Downing
N. M. Marecki
M. J. Westfall
Tech. Doc. Cntr.
Path/Tox Files

Summary

The results of the primary skin irritation test conducted from October 28, 1986 to October 31, 1986 at Riker Laboratories, Inc., St. Paul, Minnesota indicate that 50% S-26741 + 0.33% Sodium Lauryl Sulfate in Solution with Water and 5% Glycerin, is non-irritating (0.0/8.0) to the intact skin of female albino rabbits. Neither erythema nor edema were noted at any time during the study.

Introduction

The objective of this study was to determine the primary skin irritation potential of 50% S-26741 + 0.33% Sodium Lauryl Sulfate in solution with Water and 5% Glycerin, to the skin of female albino rabbits. This study was conducted for research and development purposes and is, therefore, not regulated by the Food and Drug Administration's Good Laboratory Practice Regulation of 1978, although the standard operating procedures of this laboratory adhere to the general principles of this regulation. The raw data generated by the Study Director and the final report are stored in the conducting laboratory's archives.

Animals and Husbandry

Young New Zealand White Rabbits[a] were used in the evaluation of the primary skin irritating properties of the test article. The rabbits were individually housed[b] in stainless steel cages, and food[c] and water were available ad libitum. All rabbits were individually identified with ear tags and considered to be in good health at study initiation. The rabbits were housed in a temperature and humidity controlled room. Room lighting was on a 12/12 hour light/dark cycle that was automatically timed.

Method and Results

The test procedure was modeled after that of Draize et al[d]. One day prior to the application of the test article, the hair was clipped from the back and flanks of each rabbit and two test sites selected lateral to the midline of the back approximately ten centimeters apart.

The test article (0.02 ml) was applied to one intact test site on each rabbit and immediately covered with two-inch square gauze patches. The patches, which were placed directly over the test sites, were secured with gauze wrap. The trunk of each animal was then wrapped with impervious plastic sheeting[e] which held the patches in position during the one day exposure period.

At the end of one day, the plastic wrappings, patches, and all residual test article were removed by washing with water. One hour and two days after removal of the test article, the intact test site was examined and scored separately for erythema and edema on a graded scale of 0 - 4.

The average irritation produced was evaluated by adding the mean scores for erythema and edema of the intact test sites one hour and two days post removal of the test article. These values were totaled and divided by two to obtain the mean primary irritation index.

[a] Hazleton Dutchland, Inc., Denver, PA
[b] Animals were housed in accordance with recommendations contained in DHEW Publication No. 78-23 (NIH): Revised 1985 "Guide for the Care and Use of Laboratory Animals.
[c] Purina Lab Rabbit Chow and rabbits may be offered Alfalfa Cubes for additional roughage.
[d] Draize: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965).
[e] 10 x 12 x .002 Extra Clear polyethylene sleeves, PPC Industries, Inc., Wheeling, IL

The scoring criteria for erythema and edema are shown below.

Scoring Criteria for Skin Reactions

Reaction	Description	Score
Erythema	Barely perceptible (Edges of area not defined)	1
	Pale red in color and area definable	2
	Definite red in color and area well defined.	3
	Beet or crimson red in color	4
Edema	Barely perceptible (Edges of area not defined)	1
	Area definable but not raised more than 1 mm.	2
	Area well defined and raised approximately 1 mm.	3
	Area raised more than 1 mm.	4
	Maximum Primary Irritation Score =	8

The following grading system was used to arrive at a descriptive primary skin irritation rating:

Mean Primary Irritation Score (Range of Values)	Descriptive Rating
0	Non-Irritating
0.1 - 0.5	Minimally Irritating
0.6 - 1.5	Slightly Irritating
1.6 - 3.0	Mildly Irritating
3.1 - 5.0	Moderately Irritating
5.1 - 6.5	Severely Irritating
6.6 - 8.0	Extremely Irritating

The rating for a test article may be increased if the reactions caused are beyond simple erythema and edema, e.g. necrosis, escharosis, hemorrhage. The results are presented in Table 1. The protocol, principal personnel involved in the study, composition characteristics and Quality Assurance statement are contained in Appendices I - IV.

Table 1

Primary Skin Irritation Test - Albino Rabbits

with 50% S-26741 + 0.33% Sodium Lauryl Sulfate in Solution with Water and 5% Glycerin

Animal Number	Irritation Scores for Intact Skin Sites after Removal: 1 Hour Er.	1 Hour Ed.	Day 2 Er.	Day 2 Ed.
6B1784	0	0	0	0
6B1787	0	0	0	0
6B1779	0	0	0	0
6B1782	0	0	0	0
6B1785	0	0	0	0
6B1721	0	0	0	0
Mean	0.0	0.0	0.0	0.0

Subtotal 0.0

Rating: Non-irritating

Primary Irritation Index: 0.0/8.0

Key: Er. = Erythema
Ed. = Edema

APPENDIX I
PROTOCOL

Riker Experiment No: 0386EB0638

5.

699

TEST: Acute Primary Skin Irritation Test

SPONSOR: 3M Riker Division

CONDUCTED BY: Pathology and Toxicology Department, Riker Laboratories, Inc. St. Paul, Minnesota

TEST ARTICLE: 50% S-21741 + 0.33% Sodium Lauryl Sulfate in Solution with water (5% Glycerin)

CONTROL ARTICLE: NONE

PROPOSED STARTING/COMPLETION DATE OF TEST: 10/86 - 1/87

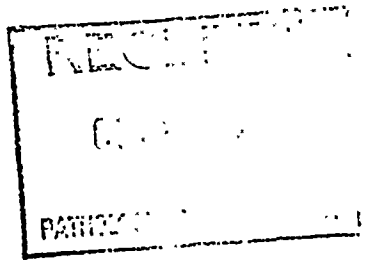
TEST SYSTEM: New Zealand White Albino Rabbits (of either sex)

SOURCE: Hazleton Dutchland, Inc., Denver, Pa.

OBJECTIVE: To determine the primary irritation potential of the test article to the skin of 6 animals. Rabbits were selected as the test system due to their historical use, sensitivity to irritants, ease of handling and general availability.

METHOD: The animals will be housed in standard wire-mesh cages in temperature and humidity controlled rooms and food[a] and water offered *ad libitum*. Each animal will be assigned a numbered ear tag, which will correspond to a card affixed to the outside of cage. Prior to the application of the test article, the hair will be clipped from the back and flanks of each animal and 1 test sites selected lateral to the midline of the back approximately ten centimeters apart. NONE of the TEST sites will be abraded by making four epidermal incisions two perpendicular to the other two, while the other test site(s) will remain intact. The test article (0.02 mL) will be applied to 0 abraded and 1 intact site(s) on each animal and secured. The trunk of each animal will then be wrapped with impervous plastic sheeting which will occlude the test article during the 1 day exposure period. One hour and 2 days after removal of the test article during the 1 day exposure period. One hour and 2 days after removal of the test article, the intact and abraded test sites will be examined and scored separately for erythema and edema on a graded scale of 0 to 4. The average irritation produced will be evaluated by adding the mean scores for erythema and edema of the intact test sites one hour and 2 days post removal of the test article. ~~Similarly, the mean scores for erythema and edema of the abraded test sites will be added~~. These two values will be totaled and divided by ~~four~~ two to obtain the mean primary irritation index and then assigned a descriptive primary skin irritation rating as follows:

Mean Primary Irritation Score	Descriptive Rating
0	Non-irritating
0.1 - 0.5	Minimally Irritating
0.6 - 1.5	Slightly Irritating
1.6 - 3.0	Midly Irritating
3.1 - 5.0	Moderately Irritating
5.1 - 6.5	Severely Irritating
6.6 - 8.0	Extremely Irritating



The rating for a test article may be increased if the reaction caused is beyond erythema and edema and are deemed to be of importance in the interpretation of the results. All raw data generated by the study director and the final report will be stored in the Riker Laboratories' Archive, St. Paul, Minnesota.

NOTE: Intact Site Only

[a] Purina Rabbit Chow, Ralston Purina Co., St. Louis, Missouri

[b] Draize: Apprasisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965) Published by the Editorial Committee of the Association of Food and Drug Officials of the United States.

Nilda R. Marcelli 10/24/86
Sponsor Date

Glen L Harris 10/27/86
Study Director Date

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APPENDIX II

Principal Participating Personnel Involved in the Study

Name	Function
G. L. Harris, B.S.	Advanced Toxicologist Study Director
G. E. Hart	Master Laboratory Technician Acute Toxicology
L. O. Wiseth	Sr. Laboratory Technician Acute Toxicology
J. A. Eads	Jr. Laboratory Technician Acute Toxicology
J. L. Allen, Ph.D.	Research Toxicologist Acute Toxicology
G. C. Pecore	Supervisor Animal Laboratory

APPENDIX III

Composition Characteristics

This study is not regulated by the Good Laboratory Practice Act of 1978 and therefore information pertaining to composition characteristics is not applicable for inclusion in this study.

APPENDIX IV

Quality Assurance Statement

This study is not officially regulated by the Good Laboratory Practice Regulation of 1978, and therefore a statement signed and prepared by the Compliance Audit department is not applicable.

The standard operating procedures of this laboratory does adhere to the general principles of this regulation. The Compliance Audit department does inspect differenct significant phases for studies underway in the Acute Toxicology Laboratory on a recurring cycle, and the facilities are examined on a three month schedule. In addition a select number of Research & Development studies are routinely picked at random from the Archives by the Compliance Audit department for review.

Pathology and Toxicology
Riker Laboratories, Inc.

Building 270-3S-05, 3M Center
St. Paul, Minnesota 55144-1000

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3M

Primary Skin Irritation Test

with 50% S-26741 + 0.35% Docusate Sodium + 0.125% N-decyl Methyl Sulfoxide in Solution with Water and 5% Glycerin

in Albino Rabbits

Riker Experiment No: 0386EB0669

Conducted At: Pathology and Toxicology
Riker Laboratories, Inc.

Dates Conducted: November 11, 1986 to November 14, 1986

Conducted By: Gene F. Harris 12/10/86
G. L. Harris, B.S. Date
Advanced Toxicologist
Study Director and Acute Toxicity
Study Coordinator

Reviewed By: C.F. Chesney (for JLA) 12-10-86
J. L. Allen, Ph.D. Date
Diplomate, A.B.T.
Research Toxicologist

dc: J. L. Allen
R. T. Catherall
C. F. Chesney
M. W. Downing
N. M. Marecki
M. J. Westfall
Tech. Doc. Cntr.
Path/Tox Files (S-26741)

Summary

The results of the primary skin irritation test conducted from November 11, 1986 to November 14, 1986 at Riker Laboratories, Inc., St. Paul, Minnesota indicate that 50% S-26741 + 0.35% Docusate Sodium + 0.125% N-decyl Methyl Sulfoxide in Solution with Water and 5% Glycerin is minimally irritating (0.1/8.0) to the intact skin of female albino rabbits. Minimal erythema in 1/6 rabbits was noted at the one hour evaluation following a one day occluded contact period. No dermal irritation was noted at the final observation on day two. The pharmacotoxic signs noted were tremors, diarrhea and salivation in 2/6 rabbits at 1 hour and tremors and diarrhea in 1/6 rabbits at 4 hours. All rabbits appeared normal at the 6 hour observation.

Introduction

The objective of this study was to determine the primary skin irritation potential of 50% S-26741 + 0.35% Docusate Sodium + 0.125% N-decyl Methyl Sulfoxide in Solution with Water and 5% Glycerin, to the skin of female albino rabbits. This study was conducted for research and development purposes and is, therefore, not regulated by the Food and Drug Administration's Good Laboratory Practice Regulation of 1978, although the standard operating procedures of this laboratory adhere to the general principles of this regulation. The raw data generated by the Study Director and the final report are stored in the conducting laboratory's archives.

Animals and Husbandry

Young New Zealand White Rabbits[a] were used in the evaluation of the primary skin irritating properties of the test article. The rabbits were individually housed[b] in stainless steel cages, and food[c] and water were available ad libitum. All rabbits were individually identified with ear tags and considered to be in good health at study initiation. The rabbits were housed in a temperature and humidity controlled room. Room lighting was on a 12/12 hour light/dark cycle that was automatically timed.

Method and Results

The test procedure was modeled after that of Draize et al[d]. One day prior to the application of the test article, the hair was clipped from the back and flanks of each rabbit and two test sites selected lateral to the midline of the back approximately ten centimeters apart.

The test article (0.02 ml) was applied to one intact test site on each rabbit and immediately covered with two-inch square gauze patches. The patches, which were placed directly over the test sites, were secured with gauze wrap. The trunk of each animal was then wrapped with impervious plastic sheeting[e] which held the patches in position during the one day exposure period.

At the end of one day, the plastic wrappings, patches, and all residual test article were removed by washing with water. One hour and two days after removal of the test article, the intact test site was examined and scored separately for erythema and edema on a graded scale of 0 - 4.

The average irritation produced was evaluated by adding the mean scores for erythema and edema of the intact test site one hour and two days post removal of the test article. These values were totaled and divided by two to obtain the mean primary irritation index.

[a] Hazleton Dutchland, Inc., Denver, PA
[b] Animals were housed in accordance with recommendations contained in DHEW Publication No. 85-23 (NIH): Revised 1985 "Guide For the Care and Use of Laboratory Animals.
[c] Purina Lab Rabbit Chow® and rabbits may be offered Alfalfa Cubes for additional roughage.
[d] Draize: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965).
[e] 10 x 12 x .002 Extra Clear polyethylene sleeves, PPC Industries, Inc., Wheeling, IL

The scoring criteria for erythema and edema are shown below.

Scoring Criteria for Skin Reactions

Reaction	Description	Score
Erythema	Barely perceptible (Edges of area not defined)	1
	Pale red in color and area definable	2
	Definite red in color and area well defined.	3
	Beet or crimson red in color	4
Edema	Barely perceptible (Edges of area not defined)	1
	Area definable but not raised more than 1 mm.	2
	Area well defined and raised approximately 1 mm.	3
	Area raised more than 1 mm.	4
	Maximum Primary Irritation Score =	8

The following grading system was used to arrive at a descriptive primary skin irritation rating:

Mean Primary Irritation Score (Range of Values)	Descriptive Rating
0	Non-Irritating
0.1 - 0.5	Minimally Irritating
0.6 - 1.5	Slightly Irritating
1.6 - 3.0	Mildly Irritating
3.1 - 5.0	Moderately Irritating
5.1 - 6.5	Severely Irritating
6.6 - 8.0	Extremely Irritating

The rating for a test article may be increased if the reactions caused are beyond simple erythema and edema, e.g. necrosis, escharosis, hemorrhage. The results are presented in Table 1. The protocol, principal personnel involved in the study, composition characteristics and Quality Assurance statement are contained in Appendices I - IV.

707

Table 1

Primary Skin Irritation Test - Albino Rabbits

with 50% S-26741 + 0.35% Docusate Sodium + 0.125% N-decyl Methyl Sulfoxide in Solution with Water and 5% Glycerin

Animal Number	Irritation Scores for Intact Skin Sites after Removal: 1 Hour Er.	1 Hour Ed.	Day 2 Er.	Day 2 Ed.
6B1836	1	0	0	0
6B1839	0	0	0	0
6B1842 [a]	0	0	0	0
6B1845	0	0	0	0
6B1837	0	0	0	0
6B1840 [a] [b]	0	0	0	0
Mean	0.2	0.0	0.0	0.0

Subtotal 0.2

Rating: Minimally irritating

Primary Irritation Index: 0.1/8.0

Key: Er. = Erythema
Ed. = Edema

[a] tremors, diarrhea, salivation, 1 hour post dose
[b] tremors, diarrhea, 4 hours post dose

All animals normal by the 6 hour observation.

APPENDIX I
PROTOCOL

Riker Experiment No: 0386EB0669

TEST: Acute Primary Skin Irritation Test 708

SPONSOR: 3M Riker Division

CONDUCTED BY: Pathology and Toxicology Department, Riker Laboratories, Inc. St. Paul, Minnesota

TEST ARTICLE: 50% S-26741 + 0.35% Decoate sodium + 0.125% n-decyl methyl sulfoxide in solution with water & 5% glycerin.

CONTROL ARTICLE: NONE

PROPOSED STARTING/COMPLETION DATE OF TEST: 11/86 - 2/87

TEST SYSTEM: New Zealand White Albino Rabbits (of either sex)

SOURCE: Hazleton Dutchland, Inc., Denver, Pa.

OBJECTIVE: To determine the primary irritation potential of the test article to the skin of 6 animals. Rabbits were selected as the test system due to their historical use, sensitivity to irritants, ease of handling and general availability.

METHOD: The animals will be housed in standard wire-mesh cages in temperature and humidity controlled rooms and food[a] and water offered *ad libitum*. Each animal will be assigned a numbered ear tag, which will correspond to a card affixed to the outside of cage. Prior to the application of the test article, the hair will be clipped from the back and flanks of each animal and 1 test sites selected lateral to the midline of the back approximately ten centimeters apart. ~~Three~~ of the TEST sites will be abraded by making four epidermal incisions two perpendicular to the other two, while the other test site(s) will remain intact. The test article (0.02 ml) will be applied to 0 abraded and 1 intact site(s) on each animal and secured. The trunk of each animal will then be wrapped with impervous plastic sheeting which will occlude the test article during the 1 day exposure period. One hour and 2 days after removal of the test article during the 1 day exposure period. One hour and 2 days after removal of the test article, the intact ~~and abraded~~ test sites will be examined and scored ~~separate~~ly for erythema and edema on a graded scale of 0 to 4. The average irritation produced will be evaluated by adding the mean scores for erythema and edema of the intact test sites one hour and 2 days post removal of the test article. ~~Similarly, the mean scores for erythema and edema of the abraded test sites will be added~~. These ~~two~~ values will be totaled and divided by ~~four~~ TWO to obtain the mean primary irritation index and then assigned a descriptive primary skin irritation rating as follows:

Mean Primary Irritation Score	Descriptive Rating
0	Non-irritating
0.1 - 0.5	Minimally Irritating
0.6 - 1.5	Slightly Irritating
1.6 - 3.0	Midly Irritating
3.1 - 5.0	Moderately Irritating
5.1 - 6.5	Severely Irritating
6.6 - 8.0	Extremely Irritating

The rating for a test article may be increased if the reaction caused is beyond erythema and edema and are deemed to be of importance in the interpretation of the results. All raw data generated by the study director and the final report will be stored in the Riker Laboratories' Archive, St. Paul, Minnesota.

[a] Purina Rabbit Chow, Ralston Purina Co., St. Louis, Missouri

[b] Draize: Apprasisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965) Published by the Editorial Committee of the Association of Food and Drug Officials of the United States.

INTACT site only

NOTE:

Sponsor 11/6/86 Date

Study Director 11/6/86 Date

Form 19171-12 F PWO

709

APPENDIX II

Principal Participating Personnel Involved in the Study

Name	Function
G. L. Harris, B.S.	Advanced Toxicologist Study Director
G. E. Hart	Master Laboratory Technician Acute Toxicology
L. O. Wiseth	Sr. Laboratory Technician Acute Toxicology
J. A. Eads	Jr. Laboratory Technician Acute Toxicology
J. L. Allen, Ph.D.	Research Toxicologist Acute Toxicology
G. C. Pecore	Supervisor Animal Laboratory

APPENDIX III

Composition Characteristics

This study is not regulated by the Good Laboratory Practice Act of 1978 and therefore information pertaining to composition characteristics is not applicable for inclusion in this study.

APPENDIX IV

Quality Assurance Statement

This study is not officially regulated by the Good Laboratory Practice Regulation of 1978, and therefore a statement signed and prepared by the Compliance Audit department is not applicable.

The standard operating procedures of this laboratory does adhere to the general principles of this regulation. The Compliance Audit department does inspect differenct significant phases for studies underway in the Acute Toxicology Laboratory on a recurring cycle, and the facilities are examined on a three month schedule. In addition a select number of Research & Development studies are routinely picked at random from the Archives by the Compliance Audit department for review.

Pathology and Toxicology
Riker Laboratories, Inc.

Building 270-3S-05, 3M Center
St. Paul, Minnesota 55144-1000

COMPANY CONFIDENTIAL

3M

Primary Skin Irritation Test

with 50% S-26741 + 0.5% Sodium Lauryl Sulfate in Solution with Water and 5% Glycerin

in Albino Rabbits

Riker Experiment No: 0386EB0670

Conducted At: Pathology and Toxicology
Riker Laboratories, Inc.

Dates Conducted: November 11, 1986 to November 14, 1986

Conducted By: Gene L. Harris 12/10/86
G. L. Harris, B.S. Date
Advanced Toxicologist
Study Director and Acute Toxicity
Study Coordinator

Reviewed By: C.F. Chesney (for JLA) 12-10-86
J. L. Allen, Ph.D. Date
Diplomate, A.B.T.
Research Toxicologist

dc: J. L. Allen
R. T. Catherall
C. F. Chesney
M. W. Downing
N. M. Marecki
M. J. Westfall
Tech. Doc. Cntr.
Path/Tox Files (S-26741)

Summary

The results of the primary skin irritation test conducted from November 11, 1986 to November 14, 1986 at Riker Laboratories, Inc., St. Paul, Minnesota indicate that 50% S-26741 + 0.5% Sodium Lauryl Sulfate in Solution with Water and 5% Glycerin, is minimally irritating (0.1/8.0) to the intact skin of female albino rabbits. Minimal erythema in 1/6 rabbits was noted at the one hour evaluation following a one day occluded contact period. No dermal irritation was noted at the final observation on day two. The pharmacotoxic signs noted during this study were tremors and diarrhea. The onset of pharmacotoxic signs occurred from 1 - 4 hours with all rabbits appearing normal by the 6 hour observation.

Introduction

The objective of this study was to determine the primary skin irritation potential of 50% S-26741 + 0.5% Sodium Lauryl Sulfate in Solution with Water and 5% Glycerin, to the skin of female albino rabbits. This study was conducted for research and development purposes and is, therefore, not regulated by the Food and Drug Administration's Good Laboratory Practice Regulation of 1978, although the standard operating procedures of this laboratory adhere to the general principles of this regulation. The raw data generated by the Study Director and the final report are stored in the conducting laboratory's archives.

Animals and Husbandry

Young New Zealand White Rabbits[a] were used in the evaluation of the primary skin irritating properties of the test article. The rabbits were individually housed[b] in stainless steel cages, and food[c] and water were available ad libitum. All rabbits were individually identified with ear tags and considered to be in good health at study initiation. The rabbits were housed in a temperature and humidity controlled room. Room lighting was on a 12/12 hour light/dark cycle that was automatically timed.

Method and Results

The test procedure was modeled after that of Draize et al[d]. One day prior to the application of the test article, the hair was clipped from the back and flanks of each rabbit and two test sites selected lateral to the midline of the back approximately ten centimeters apart.

The test article (0.02 ml) was applied to one intact test site on each rabbit and immediately covered with two-inch square gauze patches. The patches, which were placed directly over the test sites, were secured with gauze wrap. The trunk of each animal was then wrapped with impervious plastic sheeting[e] which held the patches in position during the one day exposure period.

At the end of one day, the plastic wrappings, patches, and all residual test article were removed by washing with water. One hour and two days after removal of the test article, the intact test site was examined and scored separately for erythema and edema on a graded scale of 0 - 4.

The average irritation produced was evaluated by adding the mean scores for erythema and edema of the intact test site one hour and two days post removal of the test article. These values were totaled and divided by two to obtain the mean primary irritation index.

[a] Hazleton Dutchland, Inc., Denver, PA
[b] Animals were housed in accordance with recommendations contained in DHEW Publication No. 85-23 (NIH): Revised 1985 "Guide For the Care and Use of Laboratory Animals.
[c] Purina Lab Rabbit Chow® and rabbits may be offered Alfalfa Cubes for additional roughage.
[d] Draize: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965).
[e] 10 x 12 x .002 Extra Clear polyethylene sleeves, PPC Industries, Inc., Wheeling, IL

The scoring criteria for erythema and edema are shown below.

Scoring Criteria for Skin Reactions

Reaction	Description	Score
Erythema	Barely perceptible (Edges of area not defined)	1
	Pale red in color and area definable	2
	Definite red in color and area well defined.	3
	Beet or crimson red in color	4
Edema	Barely perceptible (Edges of area not defined)	1
	Area definable but not raised more than 1 mm.	2
	Area well defined and raised approximately 1 mm.	3
	Area raised more than 1 mm.	4
	Maximum Primary Irritation Score =	8

The following grading system was used to arrive at a descriptive primary skin irritation rating:

Mean Primary Irritation Score (Range of Values)	Descriptive Rating
0	Non-Irritating
0.1 - 0.5	Minimally Irritating
0.6 - 1.5	Slightly Irritating
1.6 - 3.0	Mildly Irritating
3.1 - 5.0	Moderately Irritating
5.1 - 6.5	Severely Irritating
6.6 - 8.0	Extremely Irritating

The rating for a test article may be increased if the reactions caused are beyond simple erythema and edema, e.g. necrosis, escharosis, hemorrhage. The results are presented in Table 1. The protocol, principal personnel involved in the study, composition characteristics and Quality Assurance statement are contained in Appendices I - IV.

Table 1

Primary Skin Irritation Test - Albino Rabbits

with 50% S-26741 + 0.5% Sodium Lauryl Sulfate in Solution with Water and 5% Glycerin

Animal Number	Irritation Scores for Intact Skin Sites after Removal: 1 Hour Er.	1 Hour Ed.	Day 2 Er.	Day 2 Ed.
6B1879 [a]	1	0	0	0
6B1882	0	0	0	0
6B1874 [a] [c]	0	0	0	0
6B1877 [a]	0	0	0	0
6B1880 [a] [d]	0	0	0	0
6B1883 [b] [d]	0	0	0	0
Mean	0.2	0.0	0.0	0.0
Subtotal		0.2		

Rating: Minimally irritating

Primary Irritation Index: 0.1/8.0

Key: Er. = Erythema
Ed. = Edema

[a] tremors, 1 hour post dose
[b] tremors, diarrhea, 1 hour post dose
[c] tremors, 4 hours post dose
[d] diarrhea, 4 hours post dose

All animals normal by the 6 hour observation.

APPENDIX I Riker Experiment No: 0386EB0670
PROTOCOL

TEST: Acute Primary Skin Irritation Test 717

SPONSOR: 3M Riker Division

CONDUCTED BY: Pathology and Toxicology Department, Riker Laboratories, Inc. St. Paul, Minnesota

TEST ARTICLE: S.L.S-2674I + 0.5% Sodium Lauryl Sulfate in Solution with Water and Glycerin

CONTROL ARTICLE: NONE

PROPOSED STARTING/COMPLETION DATE OF TEST: 11/86 - 2/87

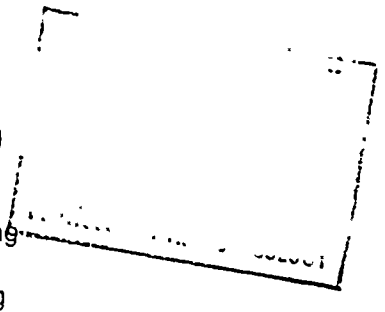
TEST SYSTEM: New Zealand White Albino Rabbits (of either sex)

SOURCE: Hazleton Dutchland, Inc., Denver, Pa.

OBJECTIVE. To determine the primary irritation potential of the test article to the skin of 6 animals. Rabbits were selected as the test system due to their historical use, sensitivity to irritants, ease of handling and general availability.

METHOD: The animals will be housed in standard wire-mesh cages in temperature and humidity controlled rooms and food[a] and water offered *ad libitum*. Each animal will be assigned a numbered ear tag. which will correspond to a card affixed to the outside of cage. Prior to the application of the test article, the hair will be clipped from the back and flanks of each animal and 1 test sites selected lateral to the midline of the back approximately ten centimeters apart. NONE of the TEST sites will be abraded by making four epidermal incisions two perpendicular to the other two, while the other test site(s) will remain intact. The test article (0.03ml) will be applied to 0 abraded and 1 intact site(s) on each animal and secured. The trunk of each animal will then be wrapped with impervous plastic sheeting which will occlude the test article during the 1 day exposure period. One hour and 2 days after removal of the test article during the 1 day exposure period. One hour and 2 days after removal of the test article, the intact ~~and abraded~~ test site~~s~~ will be examined and scored ~~separately~~ for erythema and edema on a graded scale of 0 to 4. The average irritation produced will be evaluated by adding the mean scores for erythema and edema of the intact test sites one hour and 2 days post removal of the test article. ~~Similarly, the mean scores for erythema and edema of the abraded test sites will be added~~. These ~~two~~ values will be totaled and divided by ~~four~~ two to obtain the mean primary irritation index and then assigned a descriptive primary skin irritation rating as follows:

Mean Primary Irritation Score	Descriptive Rating
0	Non-irritating
0.1 - 0.5	Minimally Irritating
0.6 - 1.5	Slightly Irritating
1.6 - 3.0	Midly Irritating
3.1 - 5.0	Moderately Irritating
5.1 - 6.5	Severely Irritating
6.6 - 8.0	Extremely Irritating



The rating for a test article may be increased if the reaction caused is beyond erythema and edema and are deemed to be of importance in the interpretation of the results. All raw data generated by the study director and the final report will be stored in the Riker Laboratories' Archive, St. Paul, Minnesota.

[a] Purina Rabbit Chow, Ralston Purina Co., St. Louis, Missouri

[b] Draize. Apprasisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965) Published by the Editorial Committee of the Association of Food and Drug Officials of the United States.

NOTE: INTACT SITE ONLY

Sponsor 11/6/86 Date

Study Director 11/6/86 Date

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APPENDIX II

Principal Participating Personnel Involved in the Study

Name	Function
G. L. Harris, B.S.	Advanced Toxicologist Study Director
G. E. Hart	Master Laboratory Technician Acute Toxicology
L. O. Wiseth	Sr. Laboratory Technician Acute Toxicology
J. A. Eads	Jr. Laboratory Technician Acute Toxicology
J. L. Allen, Ph.D.	Research Toxicologist Acute Toxicology
G. C. Pecore	Supervisor Animal Laboratory

APPENDIX III

Composition Characteristics

This study is not regulated by the Good Laboratory Practice Act of 1978 and therefore information pertaining to composition characteristics is not applicable for inclusion in this study.

APPENDIX IV

Quality Assurance Statement

This study is not officially regulated by the Good Laboratory Practice Regulation of 1978, and therefore a statement signed and prepared by the Compliance Audit department is not applicable.

The standard operating procedures of this laboratory does adhere to the general principles of this regulation. The Compliance Audit department does inspect differenct significant phases for studies underway in the Acute Toxicology Laboratory on a recurring cycle, and the facilities are examined on a three month schedule. In addition a select number of Research & Development studies are routinely picked at random from the Archives by the Compliance Audit department for review.

Pathology and Toxicology
Riker Laboratories, Inc.

Building 270-3S-05, 3M Center
St. Paul, Minnesota 55144-1000

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Primary Skin Irritation Test

with 50% S-26741 + 0.5% Sodium Myristyl Ether Sulfate in Solution with Water and 5% Glycerin

in Albino Rabbits

Riker Experiment No: 0386EB0671

Conducted At: Pathology and Toxicology
Riker Laboratories, Inc.

Dates Conducted: November 11, 1986 to November 14, 1986

Conducted By: Gene L. Harris 12/16/86
G. L. Harris, B.S. Date
Advanced Toxicologist
Study Director and Acute Toxicity
Study Coordinator

Reviewed By: C.F. Chesney (for JLA) 12-10-86
J. L. Allen, Ph.D. Date
Diplomate, A.B.T.
Research Toxicologist

dc: J. L. Allen
R. T. Catherall
C. F. Chesney
M. W. Downing
N. M. Marecki
M. J. Westfall
Tech. Doc. Cntr.
Path/Tox File (S-26741)

Summary

The results of the primary skin irritation test conducted from November 11, 1986 to November 14, 1986 at Riker Laboratories, Inc., St. Paul, Minnesota indicate that 50% S-26741 + 0.5% Sodium Myristyl Ether Sulfate in Solution with Water and 5% Glycerin, is minimally irritating (0.2/8.0) to the intact skin of female albino rabbits. Minimal erythema in 2/6 rabbits was noted at the one hour evaluation following a one day occluded contact period. No dermal irritation was noted at the final observation on day two. The pharmacotoxic signs noted during this study were tremors, diarrhea and salivation which occurred from 1 - 2 hours after dose administration. All animals appeared normal by the 4 hour observation.

Introduction

The objective of this study was to determine the primary skin irritation potential of 50% S-26741 + 0.5% Sodium Myristyl Ether Sulfate in Solution with Water and 5% Glycerin, to the skin of female albino rabbits. This study was conducted for research and development purposes and is, therefore, not regulated by the Food and Drug Administration's Good Laboratory Practice Regulation of 1978, although the standard operating procedures of this laboratory adhere to the general principles of this regulation. The raw data generated by the Study Director and the final report are stored in the conducting laboratory's archives.

Animals and Husbandry

Young New Zealand White Rabbits[a] were used in the evaluation of the primary skin irritating properties of the test article. The rabbits were individually housed[b] in stainless steel cages, and food[c] and water were available ad libitum. All rabbits were individually identified with ear tags and considered to be in good health at study initiation. The rabbits were housed in a temperature and humidity controlled room. Room lighting was on a 12/12 hour light/dark cycle that was automatically timed.

Method and Results

The test procedure was modeled after that of Draize et al[d]. One day prior to the application of the test article, the hair was clipped from the back and flanks of each rabbit and two test sites selected lateral to the midline of the back approximately ten centimeters apart.

The test article (0.02 ml) was applied to one intact test site on each rabbit and immediately covered with two-inch square gauze patches. The patches, which were placed directly over the test sites, were secured with gauze wrap. The trunk of each animal was then wrapped with impervious plastic sheeting[e] which held the patches in position during the one day exposure period.

At the end of one day, the plastic wrappings, patches, and all residual test article were removed by washing with water. One hour and two days after removal of the test article, the intact test site was examined and scored separately for erythema and edema on a graded scale of 0 - 4.

The average irritation produced was evaluated by adding the mean scores for erythema and edema of the intact test site one hour and two days post removal of the test article. These values were totaled and divided by two to obtain the mean primary irritation index.

[a] Hazleton Dutchland, Inc., Denver, PA
[b] Animals were housed in accordance with recommendations contain J in DHEW Publication No. 85-23 (NIH): Revised 1985 "Guide For the Care and Use of Laboratory Animals.
[c] Purina Lab Rabbit Chow® and rabbits may be offered Alfalfa Cubes for additional roughage.
[d] Draize: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965).
[e] 10 x 12 x .002 Extra Clear polyethylene sleeves, PPC Industries, Inc., Wheeling, IL

The scoring criteria for erythema and edema are shown below.

Scoring Criteria for Skin Reactions

Reaction	Description	Score
Erythema	Barely perceptible (Edges of area not defined)	1
	Pale red in color and area definable	2
	Definite red in color and area well defined.	3
	Beet or crimson red in color	4
Edema	Barely perceptible (Edges of area not defined)	1
	Area definable but not raised more than 1 mm.	2
	Area well defined and raised approximately 1 mm.	3
	Area raised more than 1 mm.	4
	Maximum Primary Irritation Score =	8

The following grading system was used to arrive at a descriptive primary skin irritation rating:

Mean Primary Irritation Score (Range of Values)	Descriptive Rating
0	Non-Irritating
0.1 - 0.5	Minimally Irritating
0.6 - 1.5	Slightly Irritating
1.6 - 3.0	Mildly Irritating
3.1 - 5.0	Moderately Irritating
5.1 - 6.5	Severely Irritating
6.6 - 8.0	Extremely Irritating

The rating for a test article may be increased if the reactions caused are beyond simple erythema and edema, e.g. necrosis, escharosis, hemorrhage. The results are presented in Table 1. The protocol, principal personnel involved in the study, composition characteristics and Quality Assurance statement are contained in Appendices I - IV.

Table 1

Primary Skin Irritation Test - Albino Rabbits

with 50% S-26741 + 0.5% Sodium Myristyl Ether Sulfate in Solution with Water and 5% Glycerin

Animal Number	Irritation Scores for Intact Skin Sites after Removal: 1 Hour Er.	1 Hour Ed.	Day 2 Er.	Day 2 Ed.
6B1872	1	0	0	0
6B1875 [a] [c]	0	0	0	0
6B1878 [b] [c]	0	0	0	0
6B1881 [a] [c]	0	0	0	0
6B1873 [b] [d]	0	0	0	0
6B1876 [c]	1	0	0	0
Mean	0.3	0.0	0.0	0.0
Subtotal			0.2/8.0	

Rating: Minimally irritating

Primary Irritation Index: 0.2/8.0

Key: Er. = Erythema
Ed. = Edema

[a] tremors, diarrhea, 1 hour post dose
[b] tremors, diarrhea, salivation, 1 hour post dose
[c] tremors, diarrhea, 2 hours post dose
[d] tremors, diarrhea, salivation, 2 hours post dose

All animals recovered (and appeared normal) by the 4 hour observation.

All animals normal by the 6 hour observation.

APPENDIX I
PROTOCOL

Riker Experiment No: 0386EB0671

726

TEST: Acute Primary Skin Irritation Test

SPONSOR: 3M RIKER Division

CONDUCTED BY: Pathology and Toxicology Department, Riker Laboratories, Inc. St. Paul, Minnesota

TEST ARTICLE: 50% S-26741 + 0.5% Sodium myristyl ether sulfate in solution w/water @ 5% cyclic

CONTROL ARTICLE: NONE

PROPOSED STARTING/COMPLETION DATE OF TEST: 11/86 - 2/87

TEST SYSTEM: New Zealand White Albino Rabbits (of either sex)

SOURCE: Hazleton Dutchland, Inc., Denver, Pa.

OBJECTIVE. To determine the primary irritation potential of the test article to the skin of 6 animals. Rabbits were selected as the test system due to their historical use, sensitivity to irritants, ease of handling and general availability.

METHOD. The animals will be housed in standard wire-mesh cages in temperature and humidity controlled rooms and food[a] and water offered *ad libitum*. Each animal will be assigned a numbered ear tag, which will correspond to a card affixed to the outside of cage. Prior to the application of the test article, the hair will be clipped from the back and flanks of each animal and 1 test sites selected lateral to the midline of the back approximately ten centimeters apart. NONE of the TEST sites will be abraded by making four epidermal incisions two perpendicular to the other two, while the other test site(s) will remain intact. The test article (0.02 ml) will be applied to 0 abraded and 1 intact site(s) on each animal and secured. The trunk of each animal will then be wrapped with impervous plastic sheeting which will occlude the test article during the 1 day exposure period. One hour and 2 days after removal of the test article during the 1 day exposure period. One hour and 2 days after removal of the test article, the intact ~~and abraded~~ test sites will be examined and scored separately for erythema and edema on a graded scale of 0 to 4. The average irritation produced will be evaluated by adding the mean scores for erythema and edema of the intact test sites one hour and 2 days post removal of the test article. ~~Similarly, the mean scores for erythema and edema of the abraded test sites will be added~~. These ~~two~~ values will be totaled and divided by ~~four~~ two to obtain the mean primary irritation index and then assigned a descriptive primary skin irritation rating as follows:

Mean Primary Irritation Score	Descriptive Rating
0	Non-irritating
0.1 - 0.5	Minimally Irritating
0.6 - 1.5	Slightly Irritating
1.6 - 3.0	Midly Irritating
3.1 - 5.0	Moderately Irritating
5.1 - 6.5	Severely Irritating
6.6 - 8.0	Extremely Irritating

0 = 0 SITE
NOT - INTACT

The rating for a test article may be increased if the reaction caused is beyond erythema and edema and are deemed to be of importance in the interpretation of the results. All raw data generated by the study director and the final report will be stored in the Riker Laboratories' Archive, St. Paul, Minnesota.

[a] Purina Rabbit Chow, Ralston Purina Co., St. Louis, Missouri
[b] Draize: Apprasisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965) Published by the Editorial Committee of the Association of Food and Drug Officials of the United States.

Sponsor 11/6/86 Date

Study Director 11/6/86 Date

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727

APPENDIX II

Principal Participating Personnel Involved in the Study

Name	Function
G. L. Harris, B.S.	Advanced Toxicologist Study Director
G. E. Hart	Master Laboratory Technician Acute Toxicology
L. O. Wiseth	Sr. Laboratory Technician Acute Toxicology
J. A. Eads	Jr. Laboratory Technician Acute Toxicology
J. L. Allen, Ph.D.	Research Toxicologist Acute Toxicology
G. C. Pecore	Supervisor Animal Laboratory

APPENDIX III

Composition Characteristics

This study is not regulated by the Good Laboratory Practice Act of 1978 and therefore information pertaining to composition characteristics is not applicable for inclusion in this study.

APPENDIX IV

Quality Assurance Statement

This study is not officially regulated by the Good Laboratory Practice Regulation of 1978, and therefore a statement signed and prepared by the Compliance Audit department is not applicable.

The standard operating procedures of this laboratory does adhere to the general principles of this regulation. The Compliance Audit department does inspect differenct significant phases for studies underway in the Acute Toxicology Laboratory on a recurring cycle, and the facilities are examined on a three month schedule. In addition a select number of Research & Development studies are routinely picked at random from the Archives by the Compliance Audit department for review.

Pathology and Toxicology
Riker Laboratories, Inc.

Building 270-3S-05, 3M Center
St. Paul, Minnesota 55144-1000

730

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Primary Skin Irritation Test

with 50% S-26741 + 0.5% Sodium Octyl Sulfate in Solution with Water and 5% Glycerin

in Albino Rabbits

Riker Experiment No: 0386EB0672

Conducted At: Pathology and Toxicology
Riker Laboratories, Inc.

Dates Conducted: November 11, 1986 to November 14, 1986

Conducted By: Gene L. Harris 12/10/86
G. L. Harris, B.S. Date
Advanced Toxicologist
Study Director and Acute Toxicity
Study Coordinator

Reviewed By: C.F. Chesney (for JLA) 12-10-86
J. L. Allen, Ph.D. Date
Diplomate, A.B.T.
Research Toxicologist

dc: J. L. Allen
R. T. Catherall
C. F. Chesney
M. W. Downing
N. M. Marecki
M. J. Westfall
Tech. Doc. Cntr.
Path/Tox Files (S-26741)

Summary

The results of the primary skin irritation test conducted from November 11, 1986 to November 14, 1986 at Riker Laboratories, Inc., St. Paul, Minnesota indicate that 50% S-26741 + 0.5% Sodium Octyl Sulfate in Solution with Water and 5% Glycerin, is minimally irritating (0.2/8.0) to the intact skin of female albino rabbits. Minimal erythema in 2/6 rabbits was noted at the one hour evaluation following a one day occluded contact period. No dermal irritaiton was noted at the final observation on day two. The pharmacotoxic signs noted during the study were tremors and diarrhea which occurred from 1 - 4 hours after dose administration. All animals appeared normal by the 6 hour observation.

Introduction

The objective of this study was to determine the primary skin irritation potential of 50% S-26741 + 0.5% Sodium Octyl Sulfate in Solution with Water and 5% Glycerin, to the skin of female albino rabbits. This study was conducted for research and development purposes and is, therefore, not regulated by the Food and Drug Administration's Good Laboratory Practice Regulation of 1978, although the standard operating procedures of this laboratory adhere to the general principles of this regulation. The raw data generated by the Study Director and the final report are stored in the conducting laboratory's archives.

Animals and Husbandry

Young New Zealand White Rabbits[a] were used in the evaluation of the primary skin irritating properties of the test article. The rabbits were individually housed[b] in stainless steel cages, and food[c] and water were available ad libitum. All rabbits were individually identified with ear tags and considered to be in good health at study initiation. The rabbits were housed in a temperature and humidity controlled room. Room lighting was on a 12/12 hour light/dark cycle that was automatically timed.

Method and Results

The test procedure was modeled after that of Draize et al[d]. One day prior to the application of the test article, the hair was clipped from the back and flanks of each rabbit and two test sites selected lateral to the midline of the back approximately ten centimeters apart.

The test article (0.02 ml) was applied to one intact test site on each rabbit and immediately covered with two-inch square gauze patches. The patches, which were placed directly over the test sites, were secured with gauze wrap. The trunk of each animal was then wrapped with impervious plastic sheeting[e] which held the patches in position during the one day exposure period.

At the end of one day, the plastic wrappings, patches, and all residual test article were removed by washing with water. One hour and two days after removal of the test article, the intact test site was examined and scored separately for erythema and edema on a graded scale of 0 - 4.

The average irritation produced was evaluated by adding the mean scores for erythema and edema of the intact test site one hour and two days post removal of the test article. These values were totaled and divided by two to obtain the mean primary irritation index.

[a] Hazleton Dutchland, Inc., Denver, PA
[b] Animals were housed in accordance with recommendations contained in DHEW Publication No. 85-23 (NIH): Revised 1985 "Guide For the Care and Use of Laboratory Animals.
[c] Purina Lab Rabbit Chow® and rabbits may be offered Alfalfa Cubes for additional roughage.
[d] Draize: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965).
[e] 10 x 12 x .002 Extra Clear polyethylene sleeves, PPC Industries, Inc., Wheeling, IL

The scoring criteria for erythema and edema are shown below.

Scoring Criteria for Skin Reactions

Reaction	Description	Score
Erythema	Barely perceptible (Edges of area not defined)	1
	Pale red in color and area definable	2
	Definite red in color and area well defined.	3
	Beet or crimson red in color	4
Edema	Barely perceptible (Edges of area not defined)	1
	Area definable but not raised more than 1 mm.	2
	Area well defined and raised approximately 1 mm.	3
	Area raised more than 1 mm.	4
	Maximum Primary Irritation Score =	8

The following grading system was used to arrive at a descriptive primary skin irritation rating:

Mean Primary Irritation Score (Range of Values)	Descriptive Rating
0	Non-Irritating
0.1 - 0.5	Minimally Irritating
0.6 - 1.5	Slightly Irritating
1.6 - 3.0	Mildly Irritating
3.1 - 5.0	Moderately Irritating
5.1 - 6.5	Severely Irritating
6.6 - 8.0	Extremely Irritating

The rating for a test article may be increased if the reactions caused are beyond simple erythema and edema, e.g. necrosis, escharosis, hemorrhage. The results are presented in Table 1. The protocol, principal personnel involved in the study, composition characteristics and Quality Assurance statement are contained in Appendices I - IV.

Table 1

Primary Skin Irritation Test - Albino Rabbits

50% S-26741 + 0.5% Sodium Octyl Sulfate in Solution with Water and 5% Glycerin

Animal Number	Irritation Scores for Intact Skin Sites after Removal: 1 Hour Er.	1 Hour Ed.	Day 2 Er.	Day 2 Ed.
6B1819	0	0	0	0
6B1822 [e]	1	0	0	0
6B1814 [d]	0	0	0	0
6B1817 [a] [b] [c]	0	0	0	0
6B1820 [a] [b]	1	0	0	0
6B1823	0	0	0	0
Mean	0.3	0.0	0.0	0.0

Subtotal 0.3

Rating: Minimally irritating

Primary Irritation Index: 0.2/8.0

Key: Er. = Erythema
Ed. = Edema

[a] tremors, 1 hour post dose
[b] tremors, 2 hours post dose
[c] tremors, 4 hours post dose
[d] tremors, diarrhea, 2 hours post dose
[e] tremors, diarrhea, 4 hours post dose

All animals normal by the 6 hour observation.

APPENDIX I
PROTOCOL

Riker Experiment No: 0386EB0672

TEST: Acute Primary Skin Irritation Test

SPONSOR: 3M Riker 735 Division

CONDUCTED BY: Pathology and Toxicology Department, Riker Laboratories, Inc. St. Paul, Minnesota

TEST ARTICLE: 5% S-2674 / 0.5% Sodium Octyl Sulfate in Solution with water & 5% Glycerin

CONTROL ARTICLE: NONE

PROPOSED STARTING/COMPLETION DATE OF TEST: 11/86 - 2/87

TEST SYSTEM: New Zealand White Albino Rabbits (of either sex)

SOURCE: Hazleton Dutchland, Inc., Denver, Pa.

OBJECTIVE. To determine the primary irritation potential of the test article to the skin of 6 animals. Rabbits were selected as the test system due to their historical use, sensitivity to irritants, ease of handling and general availability.

METHOD. The animals will be housed in standard wire-mesh cages in temperature and humidity controlled rooms and food[a] and water offered *ad libitum*. Each animal will be assigned a numbered ear tag, which will correspond to a card affixed to the outside of cage. Prior to the application of the test article, the hair will be clipped from the back and flanks of each animal and 1 test sites selected lateral to the midline of the back approximately ten centimeters apart. NONE of the TEST sites will be abraded by making four epidermal incisions two perpendicular to the other two, while the other test site(s) will remain intact. The test article (0.02 ml) will be applied to 0 abraded and 1 intact site(s) on each animal and secured. The trunk of each animal will then be wrapped with impervous plastic sheeting which will occlude the test article during the 1 day exposure period. One hour and 2 days after removal of the test article during the 1 day exposure period. One hour and 2 days after removal of the test article, the intact ~~and abraded~~ test site~~s~~ will be examined and scored separately for erythema and edema on a graded scale of 0 to 4. The average irritation produced will be evaluated by adding the mean scores for erythema and edema of the intact test sites one hour and 2 days post removal of the test article. ~~Similarly, the mean scores for erythema and edema of the abraded test sites will be added.~~ These ~~two~~ values will be totaled and divided by ~~four~~ two to obtain the mean primary irritation index and then assigned a descriptive primary skin irritation rating as follows:

Mean Primary Irritation Score	Descriptive Rating
0	Non-irritating
0.1 - 0.5	Minimally Irritating
0.6 - 1.5	Slightly Irritating
1.6 - 3.0	Midly Irritating
3.1 - 5.0	Moderately Irritating
5.1 - 6.5	Severely Irritating
6.6 - 8.0	Extremely Irritating

NOTE: INTACT SITE ONLY

The rating for a test article may be increased if the reaction caused is beyond erythema and edema and are deemed to be of importance in the interpretation of the results. All raw data generated by the study director and the final report will be stored in the Riker Laboratories' Archive. St. Paul, Minnesota.

[a] Purina Rabbit Chow, Ralston Purina Co., St. Louis, Missouri

[b] Draize: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965) Published by the Editorial Committee of the Association of Food and Drug Officials of the United States.

Sponsor 11/6/86 Date

Study Director 11/6/86 Date

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APPENDIX II

Principal Participating Personnel Involved in the Study

Name	Function
G. L. Harris, B.S.	Advanced Toxicologist Study Director
G. E. Hart	Master Laboratory Technician Acute Toxicology
L. O. Wiseth	Sr. Laboratory Technician Acute Toxicology
J. A. Eads	Jr. Laboratory Technician Acute Toxicology
J. L. Allen, Ph.D.	Research Toxicologist Acute Toxicology
G. C. Pecore	Supervisor Animal Laboratory

APPENDIX III

Composition Characteristics

This study is not regulated by the Good Laboratory Practice Act of 1978 and therefore information pertaining to composition characteristics is not applicable for inclusion in this study.

APPENDIX IV

Quality Assurance Statement

This study is not officially regulated by the Good Laboratory Practice Regulation of 1978, and therefore a statement signed and prepared by the Compliance Audit department is not applicable.

The standard operating procedures of this laboratory does adhere to the general principles of this regulation. The Compliance Audit department does inspect differenct significant phases for studies underway in the Acute Toxicology Laboratory on a recurring cycle, and the facilities are examined on a three month schedule. In addition a select number of Research & Development studies are routinely picked at random from the Archives by the Compliance Audit department for review.

Pathology and Toxicology
Riker Laboratories, Inc.

Building 270-3S-05, 3M Center
St Paul, Minnesota 55144-1000

739

COMPANY CONFIDENTIAL

3M

Repeat Skin Irritation Test

with Hydroxypropylmethylcellulose Gel Containing

50% S-26741, Lot FN4588

in Albino Guinea Pigs

Riker Experiment No: 0387EG0053

Conducted At: Pathology and Toxicology
Riker Laboratories, Inc.

Dates Conducted: March 4, 1987 to March 25, 1987

Conducted By: 12/16/87
G. E. Hart Date
Acute Toxicity Study Coordinator
Study Director

Reviewed By: 12-17-87
J. L. Allen, Ph.D. Date
Diplomate, A.B.T.
Research Toxicologist

dc: J.L. Allen
R.T. Catherall
M.W. Downing
N.M. Marecki
M.J. Westfall
Path Tox Files

Summary

The results of the repeat skin irritation test conducted from March 4, 1987 to March 25, 1987 at Riker Laboratories, Inc., St. Paul, Minnesota indicate that Hydroxypropylmethylcellulose gel containing 50% S-26741, Lot FN4588, is practically non-irritating to the skin of female albino guinea pigs using a different naive test site for each of the nine applications. Minimal erythema was noted at the 5th (1/10 guinea pigs) and 9th (2/10 guinea pigs) applications following a 24 hour occluded contact period.

Introduction

The objective of this study was to determine the repeat skin irritation potential of Hydroxypropylmethylcellulose gel containing 50% S-26741, Lot FN4588, to the skin of female albino guinea pigs. This study was conducted in accordance with the Food and Drug Administration's Good Laboratory Practice Regulation of 1978. The raw data generated by the Study Director and the final report are stored in the conducting laboratory's archives.

Animals and Husbandry

Young Hartley Albino Guinea Pigs[a] were used in the evaluation of the repeat skin irritating properties of the test article. The guinea pigs were individually housed[b] in stainless steel cages, and food[c] and water were available ad libitum. All guinea pigs were individually identified and considered to be in good health at study initiation. The guinea pigs were housed in a temperature and humidity controlled room. Room lighting was on a 12/12 hour light/dark cycle that was automatically timed.

Method and Results

Prior to each of the nine applications, the hair was clipped from a naive test site on the trunk of each guinea pig.

The test article was applied 3 times per week for 3 weeks (Monday, Wednesday, Friday) to the skin. The test article (0.1 ml) on a patch[d], was applied to each test site on each guinea pig. The patches were applied to the trunk of each animal and then wrapped with gauze and secured with elastic bandage[e] which occluded the test article during the 24 hour contact period.

At the end of the exposure period the wrappings and all residual test article were removed manually. Twenty-four hours after removal of the test article, the test sites were examined and scored separately for erythema and edema on a graded scale of 0 - 4.

The scoring criteria for erythema and edema are shown below.

Scoring Criteria for Skin Reactions

Reaction	Description	Score
Erythema	Barely perceptible (Edges of area not defined)	1
	Pale red in color and area definable	2
	Definite red in color and area well defined.	3
	Beet or crimson red in color	4
Edema	Barely perceptible (Edges of area not defined)	1
	Area definable but not raised more than 1 mm.	2
	Area well defined and raised approximately 1 mm.	3
	Area raised more than 1 mm.	4
	Maximum Primary Irritation Score =	8

The results are presented in Table 1. The protocol, principal personnel involved in the study, composition characteristics and Quality Assurance statement are contained in Appendices I - IV.

[a] Charles River Breeding Labs, Wilmington, MA
[b] Animals were housed in accordance with recommendations contained in DHEW Publication No. 85-23 (NIH): Revised 1985 "Guide For the Care and Use of Laboratory Animals."
[c] Purina Lab Guinea Pig Chow, Ralston Purina, St. Louis, MO
[d] Readi Bandage, Park Davis Co., Detroit, MI 48232
[e] Elastoplast, Beiersdorf, Inc. South Norwalk, CT

Table 1

Repeat Skin Irritation Test - Albino Guinea Pigs

with Hydroxypropylmethylcellulose Gel
Containing 50% S-26741, Lot FN4588

1 Hour
Irritation Scores for Intact
Skin Sites after Removal:

Animal Number	Dose 1		Dose 2		Dose 3		Dose 4		Dose 5	
	ER.	ED.	ER.	ED.	ER.	ED.	ER.	ED.	ER.	ED.
7G809	0	0	0	0	0	0	0	0	1	0
7G815	0	0	0	0	0	0	0	0	0	0
7G821	0	0	0	0	0	0	0	0	0	0
7G827	0	0	0	0	0	0	0	0	0	0
7G833	0	0	0	0	0	0	0	0	0	0
7G810	0	0	0	0	0	0	0	0	0	0
7G816	0	0	0	0	0	0	0	0	0	0
7G822	0	0	0	0	0	0	0	0	0	0
7G828	0	0	0	0	0	0	0	0	0	0
7G834	0	0	0	0	0	0	0	0	0	0

Animal Number	Dose 6		Dose 7		Dose 8		Dose 9	
	ER.	ED.	ER.	ED.	ER.	ED.	ER.	ED.
7G809	0	0	0	0	0	0	1	0
7G815	0	0	0	0	0	0	0	0
7G821	0	0	0	0	0	0	0	0
7G827	0	0	0	0	0	0	0	0
7G833	0	0	0	0	0	0	0	0
7G810	0	0	0	0	0	0	1	0
7G816	0	0	0	0	0	0	0	0
7G822	0	0	0	0	0	0	0	0
7G828	0	0	0	0	0	0	1	0
7G834	0	0	0	0	0	0	0	0

Key: ER. = Erythema
ED. = Edema

APPENDIX I

PROTOCOL

TEST: Skin Irritation Test (Repeat Application) 743

SPONSOR: 3M RIKER Division

CONDUCTED BY: **Safety Evaluation Laboratory, Riker Laboratories, Inc., St. Paul, Minnesota**

TEST ARTICLE: Hydroxypropyltrimethylammonium Guar Chloride 50% w/w Pyridoxine Peemide, FN 4958

CONTROL ARTICLE: None

PROPOSED STARTING/COMPLETION DATE OF TEST: 2/87 - 6/87

TEST SYSTEM: Female Hartley Albino Guinea Pigs

SOURCE: Hazleton-Dutchland, Denver, PA

OBJECTIVE: To assess the irritation potential of the test article to the skin of guinea pigs after repeat contact. Guinea pigs were selected as the test system due to their historical use, sensitivity to irritants, and so that a direct comparison of irritation can be made available for guinea pig skin sensitization studies that will be conducted concurrently.

METHOD: The animals will be housed in standard wire-mesh cages in temperature and humidity controlled rooms with food[a] and water offered ad libitum. Each animal will be assigned a numbered ear tag, which will correspond to a card affixed to the outside of the cage.

Test article administration will consist of nine topical applications of the test article[b] at three applications per week (Monday, Wednesday and Friday) at a naive test site for each of the nine applications. Each test site will be clipped free of hair prior to the application procedure. The test article will be placed on each animal and firmly secured. The test article will be left in place for approximately 24 hours, after which all residual test material will be removed. Each animal will be evaluated[c] for signs of skin irritation approximately 24 hours after removal of the test article for each of the nine exposures. The daily scores for erythema and edema will be meaned and assessed for potential cumulative irritation.

[a] Purina Guinea Pig Chow, Ralston Purina Co., St. Louis, Missouri
[b] The test article dose will be 0.1 ml for each application.
[c] Draize: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965)

Marie Wolfgang 2/27/87 Glen L. Harris 3/2/87

Sponsor Date Study Director Date

Riker Experiment No. 0387EG0053

Appendix I (concluded)
Deviations and/or Amendments to Protocol

1. The source of animals used in this study is Charles River Breeding Labs, Wilmington, MA

   Reason for change: incorrect source typed on original protocol.

   Gene L. Harris — Study Director — 3/2/87 — Date

2. Ten animals were used for this study.

   Reason for change: animal number was omitted from the original protocol.

   Gene L. Harris — Study Director — 3/11/87 — Date

3. The completion date will be extended to 12/87 due to delays in report preparation.

   Jerry Hart — Study Director — 12/16/87 — Date

4. The erythema and edema will not be meaned.

   Reason for change: Not required to interpret test.

   Jerry Hart — Study Director — 12/17/87 — Date

APPENDIX II

Principal Participating Personnel Involved in the Study

Name	Function
G. E. Hart	Master Laboratory Technician Study Director
L. O. Wiseth	Sr. Laboratory Technician Acute Toxicology
J. L. Allen, Ph.D.	Research Toxicologist Acute Toxicology
J. A. Eads	Junior Laboratory Technician Acute Toxicology
K. A. Moore	Junior Laboratory Technician Acute Toxicology
G. C. Pecore	Supervisor Animal Laboratory

APPENDIX III

Test and/or Control Article Characterization

for

Hydroxypropylmethylcellulose gel containing 50% w/w pyridostigmine Bromide (FN 4588)

1. The identity strength, uniformity, composition, purity or other pertinent characterizations of the test and/or substances have been determined and documented as of RFA 14203 - 2/27/87.

2. The method of synthesis or origin of the test and control substances, including their amount and the method of bioassay (if applicable) is documented.

   ☒ Yes ☐ No *Amit a Patel 2/27/87

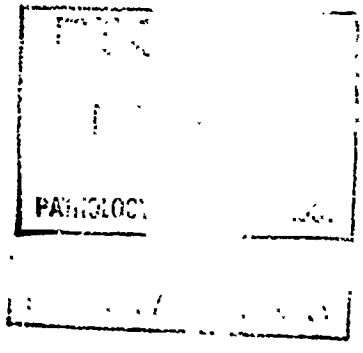
3. The stability of the test and/or control substances ~~have been determined~~ ~~or~~ will be determined ~~as of~~* at end of study.
   AT Amit a Patel (2/2/87)

The above information and documentation are located in the sponsor's records.

~~Sponsor or~~ Sponsor Representative	Date
Amit a Patel	2/27/87

Original characterization can be found in experiment no 0387MG0051.

* = Form CHANGE



APPENDIX IV

QUALITY ASSURANCE STATEMENT

Acute Toxicology Laboratory Studies

Study No.: 0387EG0053

This short term study was audited by Compliance Audit and the final report examined against the raw data on December 18, 1987. The results of the audit were reported to the study director and to management on December 18, 1987.

In addition to the data audit, different significant phases for studies underway in the Acute Toxicology Laboratory are inspected on a recurring cycle, and the facilities are examined by Compliance Audit on a yearly schedule.

D. M. Marbo, Jr
Compliance Audit

12-18-87
Date

Pathology and Toxicology
Riker Laboratories, Inc.

Building 270-3S-05, 3M Center
St. Paul, Minnesota 55144-1000

COMPANY CONFIDENTIAL

3M

Repeat Skin Irritation Test

with Hydroxypropylmethylcellulose Gel Containing

30% S-26741 + 0.21% Docusate Sodium, Lot FN4589

in Albino Guinea Pigs

Riker Experiment No:	0387EG0056
Conducted At:	Pathology and Toxicology Riker Laboratories, Inc.
Dates Conducted:	March 4, 1987 to March 25, 1987
Conducted By:	G E Hart 12/17/87 G. E. Hart Date Acute Toxicity Study Coordinator Study Director
Reviewed By:	J L Allen 12-17-87 J. L. Allen, Ph.D. Date Diplomate, A.B.T. Research Toxicologist

dc: [redacted]
R.T. Catherall
M.W. Downing
N.M. Marecki
M.J. Westfall
Path Tox Files

Summary

The results of the repeat skin irritation test conducted from March 4, 1987 to March 25, 1987 at Riker Laboratories, Inc., St. Paul, Minnesota indicate that Hydroxypropylmethylcellulose gel containing 30% S-26741 and 0.21% docusate sodium, Lot FN4589, is practically non-irritating to the skin of female albino guinea pigs using a different naive test site for each of the nine applications. Minimal erythema was noted at the 6th (1/10 guinea pigs), 8th (1/10 guinea pigs), and 9th (2/10 guinea pigs) applications following a 24 hour occluded contact period.

Introduction

The objective of this study was to determine the repeat skin irritation potential of Hydroxypropylmethylcellulose gel containing 30% S-26741 and 0.21% docusate sodium, Lot FN4589, to the skin of female albino guinea pigs. This study was conducted in accordance with the Food and Drug Administration's Good Laboratory Practice Regulation of 1978. The raw data generated by the Study Director and the final report are stored in the conducting laboratory's archives.

Animals and Husbandry

Young Hartley Albino Guinea Pigs[a] were used in the evaluation of the repeat skin irritating properties of the test article. The guinea pigs were individually housed[b] in stainless steel cages, and food[c] and water were available ad libitum. All guinea pigs were individually identified and considered to be in good health at study initiation. The guinea pigs were housed in a temperature and humidity controlled room. Room lighting was on a 12/12 hour light/dark cycle that was automatically timed.

Method and Results

Prior to each of the nine applications, the hair was clipped from a naive test site on the trunk of each guinea pig.

The test article was applied 3 times per week for 3 weeks (Monday, Wednesday, Friday) to the skin. The test article (0.1 ml) on a patch[d], was applied to each test site on each guinea pig. The patches were applied to the trunk of each animal and then wrapped with gauze and secured with elastic bandage[e] which occluded the test article during the 24 hour contact period.

At the end of the exposure period the wrappings and all residual test article were removed manually. Twenty-four hours after removal of the test article, the test sites were examined and scored separately for erythema and edema on a graded scale of 0 - 4.

The scoring criteria for erythema and edema are shown below.

Scoring Criteria for Skin Reactions

Reaction	Description	Score
Erythema	Barely perceptible (Edges of area not defined)	1
	Pale red in color and area definable	2
	Definite red in color and area well defined.	3
	Beet or crimson red in color	4
Edema	Barely perceptible (Edges of area not defined)	1
	Area definable but not raised more than 1 mm.	2
	Area well defined and raised approximately 1 mm.	3
	Area raised more than 1 mm.	4
	Maximum Primary Irritation Score =	8

The results are presented in Table 1. The protocol, principal personnel involved in the study, composition characteristics and Quality Assurance statement are contained in Appendices I - IV.

[a] Charles River Breeding Labs, Wilmington, MA

[b] Animals were housed in accordance with recommendations contained in DHEW Publication No. 85-23 (NIH): Revised 1985 "Guide For the Care and Use of Laboratory Animals."

[c] Purina Lab Guinea Pig Chow, Ralston Purina, St. Louis, MO

[d] Readi Bandage, Park Davis Co., Detroit, MI 48232

[e] Elastoplast, Beiersdorf, Inc. South Norwalk, CT

Table 1

Repeat Skin Irritation Test - Albino Guinea Pigs

with Hydroxypropylmethylcellulose Gel
Containing 30% S-26741 and 0.21% Docusate Sodium, Lot FN4589

1 Hour
Irritation Scores for Intact
Skin Sites after Removal:

Animal Number	Dose 1		Dose 2		Dose 3		Dose 4		Dose 5	
	ER.	ED.	ER.	ED.	ER.	ED.	ER.	ED.	ER.	ED.
7G811	0	0	0	0	0	0	0	0	0	0
7G817	0	0	0	0	0	0	0	0	0	0
7G823	0	0	0	0	0	0	0	0	0	0
7G829	0	0	0	0	0	0	0	0	0	0
7G835	0	0	0	0	0	0	0	0	0	0
7G812	0	0	0	0	0	0	0	0	0	0
7G818	0	0	0	0	0	0	0	0	0	0
7G824	0	0	0	0	0	0	0	0	0	0
7G830	0	0	0	0	0	0	0	0	0	0
7G836	0	0	0	0	0	0	0	0	0	0

Animal Number	Dose 6		Dose 7		Dose 8		Dose 9	
	ER.	ED.	ER.	ED.	ER.	ED.	ER.	ED.
7G811	0	0	0	0	0	0	1	0
7G817	0	0	0	0	0	0	0	0
7G823	0	0	0	0	0	0	0	0
7G829	0	0	0	0	0	0	0	0
7G835	0	0	0	0	0	0	0	0
7G812	0	0	0	0	1	0	1	0
7G818	0	0	0	0	0	0	0	0
7G824	1	0	0	0	0	0	0	0
7G830	0	0	0	0	0	0	0	0
7G836	0	0	0	0	0	0	0	0

Key: ER. = Erythema
ED. = Edema

APPENDIX I

PROTOCOL

TEST: Skin Irritation Test (Repeat Application) 752

SPONSOR: 3M RIKER Division

CONDUCTED BY: **Safety Evaluation Laboratory, Riker Laboratories, Inc., St. Paul, Minnesota**

TEST ARTICLE: Hydroxypropylmethylcellulose gel containing 30% Pyridostigmine Bromide and 0.21% Decoate Sodium, Lot FN 4589

CONTROL ARTICLE: None

PROPOSED STARTING/COMPLETION DATE OF TEST: 2/87 - 6/87

TEST SYSTEM: Female Hartley Albino Guinea Pigs

SOURCE: Hazleton-Dutchland, Denver, PA

OBJECTIVE: To assess the irritation potential of the test article to the skin of guinea pigs after repeat contact. Guinea pigs were selected as the test system due to their historical use, sensitivity to irritants, and so that a direct comparison of irritation can be made available for guinea pig skin sensitization studies that will be conducted concurrently.

METHOD: The animals will be housed in standard wire-mesh cages in temperature and humidity controlled rooms with food[a] and water offered ad libitum. Each animal will be assigned a numbered ear tag, which will correspond to a card affixed to the outside of the cage.

Test article administration will consist of nine topical applications of the test article[b] at three applications per week (Monday, Wednesday and Friday) at a naive test site for each of the nine applications. Each test site will be clipped free of hair prior to the application procedure. The test article will be placed on each animal and firmly secured. The test article will be left in place for approximately 24 hours, after which all residual test material will be removed. Each animal will be evaluated[c] for signs of skin irritation approximately 24 hours after removal of the test article for each of the nine exposures. The daily scores for erythema and edema will be meaned and assessed for potential cumulative irritation.

[a] Purina Guinea Pig Chow, Ralston Purina Co., St. Louis, Missouri
[b] The test article dose will be 0.1 ml for each application.
[c] Draize: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965)

Marie Westfall 2/27/87
Sponsor Date

Glenn L Harris 3/2/87
Study Director Date

Riker Experiment No. 0387EG0056

Appendix I (concluded)
Deviations and/or Amendments to Protocol

1. The source of animals used in this study is Charles River Breeding Labs, Wilmington, MA

   Reason for change: incorrect source typed on original protocol.

   Gene L. Harris — Study Director — 3/2/87 Date

2. Ten animals were used for this study.

   Reason for change: omitted from the original protocol.

   Gene L. Harris — Study Director — 3/11/87 Date

3. The completion date will be extended to 12/87 due to report preparation delays.

   Jerry Hart — Study Director — 12/16/87 Date

4. The erythema and edema score will not be meaned.

   Reason for change: Not required to interpret test results.

   Jerry Hart — Study Director — 12/17/87 Date

APPENDIX II

Principal Participating Personnel Involved in the Study

Name	Function
G. E. Hart	Master Laboratory Technician Study Director
L. O. Wiseth	Sr. Laboratory Technician Acute Toxicology
J. L. Allen, Ph.D.	Research Toxicologist Acute Toxicology
J. A. Eads	Junior Laboratory Technician Acute Toxicology
K. A. Moore	Junior Laboratory Technician Acute Toxicology
G. C. Pecore	Supervisor Animal Laboratory

APPENDIX III

Test and/or Control Article Characterization

for

Hydroxypropylmethylcellulose gel containing 30% pyridostigmine Brom and 0.2% Docusate Sodium (FN 4589)

1. The identity strength, uniformity, composition, purity or other pertinent characterizations of the test and/or substances have been determined and documented as of RFA 14201 – 2/27/87.

2. The method of synthesis or origin of the test and control substances, including their amount and the method of bioassay (if applicable) is documented. FN4589

   ☒ Yes ☐ No Anita Tilton 2/27/87

3. The stability of the test and/or control substances ~~have been determined or~~ will be determined ~~as~~ AT of * the end of the study.

   Anita Tilton 2/27/87

The above information and documentation are located in the sponsor's records.

~~Sponsor or~~ Sponsor Representative	Date
Anita Tilton	2/27/87

Original characterization can be found in E/P # 0387MB.0054

* = Form change

APPENDIX IV

QUALITY ASSURANCE STATEMENT

Acute Toxicology Laboratory Studies

Study No.: 0387EG0056

This short term study was audited by Compliance Audit and the final report examined against the raw data on December 17, 1987. The results of the audit were reported to the study director and to management on December 17, 1987.

In addition to the data audit, different significant phases for studies underway in the Acute Toxicology Laboratory are inspected on a recurring cycle, and the facilities are examined by Compliance Audit on a ~~three month~~ yearly 12-17-87 schedule.

D. M. Markee, D

Compliance Audit

12-17-87

Date

Pathology and Toxicology
Riker Laboratories, Inc.

Building 270-3S-05, 3M Center
St. Paul, Minnesota 55144-1000

757

COMPANY CONFIDENTIAL

3M

Repeat Skin Irritation Test

with Hydroxypropylmethylcellulose Gel Containing

30% S-26741 + 0.198% Sodium Lauryl Sulfate, Lot FN4590

in Albino Guinea Pigs

Riker Experiment No: 0387EG0059

Conducted At: Pathology and Toxicology
Riker Laboratories, Inc.

Dates Conducted: March 4, 1987 to March 25, 1987

Conducted By: G. E. Hart 12/16/87
G. E. Hart Date
Acute Toxicity Study Coordinator
Study Director

Reviewed By: J. L. Allen 12-17-87
J. L. Allen, Ph.D. Date
Diplomate, A.B.T.
Research Toxicologist

dc: J.L. Allen
R.T. Catherall
M.W. Downing
N.M. Marecki
M.J. Westfall
Path Tox Files

Summary

The results of the repeat skin irritation test conducted from March 4, 1987 to March 25, 1987 at Riker Laboratories, Inc., St. Paul, Minnesota indicate that Hydroxypropylmethylcellulose gel containing 30% S-26741 and 0.198% sodium lauryl sulfate, Lot FN4590, is practically non-irritating to the skin of female albino guinea pigs using a different naive test site for each of the nine applications. Minimal erythema was noted at the 8th (3/10 guinea pigs), and 9th (2/10 guinea pigs) applications following a 24 hour occluded contact period.

Introduction

The objective of this study was to determine the repeat skin irritation potential of Hydroxypropylmethylcellulose gel containing 30% S-26741 and 0.198% sodium lauryl sulfate, Lot FN4590, to the skin of female albino guinea pigs. This study was conducted in accordance with the Food and Drug Administration's Good Laboratory Practice Regulation of 1978. The raw data generated by the Study Director and the final report are stored in the conducting laboratory's archives.

Animals and Husbandry

Young Hartley Albino Guinea Pigs[a] were used in the evaluation of the repeat skin irritating properties of the test article. The guinea pigs were individually housed[b] in stainless steel cages, and food[c] and water were available ad libitum. All guinea pigs were individually identified and considered to be in good health at study initiation. The guinea pigs were housed in a temperature and humidity controlled room. Room lighting was on a 12/12 hour light/dark cycle that was automatically timed.

Method and Results

Prior to each of the nine applications, the hair was clipped from a naive test site on the trunk of each guinea pig.

The test article was applied 3 times per week for 3 weeks (Monday, Wednesday, Friday) to the skin. The test article (0.1 ml) on a patch[d], was applied to each test site on each guinea pig. The patches were applied to the trunk of each animal and then wrapped with gauze and secured with elastic bandage[e] which occluded the test article during the 24 hour contact period.

At the end of the exposure period the wrappings and all residual test article were removed manually. Twenty-four hours after removal of the test article, the test sites were examined and scored separately for erythema and edema on a graded scale of 0 - 4.

The scoring criteria for erythema and edema are shown below.

Scoring Criteria for Skin Reactions

Reaction	Description	Score
Erythema	Barely perceptible (Edges of area not defined)	1
	Pale red in color and area definable	2
	Definite red in color and area well defined.	3
	Beet or crimson red in color	4
Edema	Barely perceptible (Edges of area not defined)	1
	Area definable but not raised more than 1 mm.	2
	Area well defined and raised approximately 1 mm.	3
	Area raised more than 1 mm.	4
	Maximum Primary Irritation Score =	8

The results are presented in Table 1. The protocol, principal personnel involved in the study, composition characteristics and Quality Assurance statement are contained in Appendices I - IV.

[a] Charles River Breeding Labs, Wilmington, MA
[b] Animals were housed in accordance with recommendations contained in DHEW Publication No. 85-23 (NIH): Revised 1985 "Guide For the Care and Use of Laboratory Animals."
[c] Purina Lab Guinea Pig Chow, Ralston Purina, St. Louis, MO
[d] Readi Bandage, Park Davis Co., Detroit, MI 48232
[e] Elastoplast, Beiersdorf, Inc. South Norwalk, CT

Table 1

Repeat Skin Irritation Test - Albino Guinea Pigs

with Hydroxypropylmethylcellulose Gel
Containing 30% S-26741 and 0.198% Sodium Lauryl Sulfate,
Lot FN4590

1 Hour
Irritation Scores for Intact
Skin Sites after Removal:

Animal Number	Dose 1		Dose 2		Dose 3		Dose 4		Dose 5	
	ER.	ED.	ER.	ED.	ER.	ED.	ER.	ED.	ER.	ED.
7G813	0	0	0	0	0	0	0	0	0	0
7G819	0	0	0	0	0	0	0	0	0	0
7G825	0	0	0	0	0	0	0	0	0	0
7G831	0	0	0	0	0	0	0	0	0	0
7G837	0	0	0	0	0	0	0	0	0	0
7G814	0	0	0	0	0	0	0	0	0	0
7G820	0	0	0	0	0	0	0	0	0	0
7G826	0	0	0	0	0	0	0	0	0	0
7G832	0	0	0	0	0	0	0	0	0	0
7G838	0	0	0	0	0	0	0	0	0	0

Animal Number	Dose 6		Dose 7		Dose 8		Dose 9	
	ER.	ED.	ER.	ED.	ER.	ED.	ER.	ED.
7G813	0	0	0	0	1	0	0	0
7G819	0	0	0	0	0	0	0	0
7G825	0	0	0	0	0	0	0	0
7G831	0	0	0	0	1	0	0	0
7G837	0	0	0	0	0	0	0	0
7G814	0	0	0	0	1	0	0	0
7G820	0	0	0	0	0	0	0	0
7G826	0	0	0	0	0	0	1	0
7G832	0	0	0	0	0	0	1	0
7G838	0	0	0	0	0	0	0	0

Key: ER. = Erythema
ED. = Edema

RIKER Experiment No.: 0387EG0059

APPENDIX I
PROTOCOL

4.

TEST: Skin Irritation Test (Repeat Application)

761

SPONSOR: 3M RIKER Division

CONDUCTED BY: **Safety Evaluation Laboratory, Riker Laboratories, Inc., St. Paul, Minnesota**

TEST ARTICLE: Pilocarpine Base 0.5% And 0.19% Sodium Lauryl Sulfate, Lot FN 459C

CONTROL ARTICLE: None

PROPOSED STARTING/COMPLETION DATE OF TEST: 2/87 - 6/87

TEST SYSTEM: Female Hartley Albino Guinea Pigs

SOURCE: Hazleton-Dutchland, Denver, PA

OBJECTIVE: To assess the irritation potential of the test article to the skin of guinea pigs after repeat contact. Guinea pigs were selected as the test system due to their historical use, sensitivity to irritants, and so that a direct comparison of irritation can be made available for guinea pig skin sensitization studies that will be conducted concurrently.

METHOD: The animals will be housed in standard wire-mesh cages in temperature and humidity controlled rooms with food[a] and water offered ad libitum. Each animal will be assigned a numbered ear tag, which will correspond to a card affixed to the outside of the cage.

Test article administration will consist of nine topical applications of the test article[b] at three applications per week (Monday, Wednesday and Friday) at a naive test site for each of the nine applications. Each test site will be clipped free of hair prior to the application procedure. The test article will be placed on each animal and firmly secured. The test article will be left in place for approximately 24 hours, after which all residual test material will be removed. Each animal will be evaluated[c] for signs of skin irritation approximately 24 hours after removal of the test article for each of the nine exposures. The daily scores for erythema and edema will be meaned and assessed for potential cumulative irritation.

[a] Purina Guinea Pig Chow, Ralston Purina Co., St. Louis, Missouri
[b] The test article dose will be 0.1 ml for each application.
[c] Draize: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965)

Sponsor 2/27/87 Date — Study Director 3/6/87 Date

Riker Experiment No. 0387EG0059

Appendix I (concluded)
Deviations and/or Amendments to Protocol

1. The source of animals used in this study is Charles River Breeding Labs, Wilmington, MA

Reason for change: incorrect source typed on original protocol.

Gene L. Harris — Study Director — 3/2/87 — Date

2. Ten animals were used for this study.

Reason for change: omitted from the original protocol.

Gene L. Harris — Study Director — 3/11/87 — Date

3. The completion date will be extended to 12/87 due to report preparation delays.

Jerry Hart — Study Director — 12/16/87 — Date

4. The erythema and edema will not be meaned.

Reason for change: Not required to interpret test.

Jerry Hart — Study Director — 12/17/87 — Date

APPENDIX II

Principal Participating Personnel Involved in the Study

Name	Function
G. E. Hart	Master Laboratory Technician Study Director
L. O. Wiseth	Sr. Laboratory Technician Acute Toxicology
J. L. Allen, Ph.D.	Research Toxicologist Acute Toxicology
J. A. Eads	Junior Laboratory Technician Acute Toxicology
K. A. Moore	Junior Laboratory Technician Acute Toxicology
G. C. Pecore	Supervisor Animal Laboratory

APPENDIX III

Test and/or Control Article Characterization

for

Hydroxypropyl methylcellulose gel containing 30% pyridoxamine Br. and 0.198% Sodium Lauryl Sulfate, (FN 4590)

1. The identity strength, uniformity, composition, purity or other pertinent characterizations of the test and/or substances have been determined and documented as of RFA 14202-2/27/87.

2. The method of synthesis or origin of the test and control substances, including their amount and the method of bioassay (if applicable) is documented.

☐ Yes ☐ No Analytical 2/27/87 *

3. The stability of the test and/or control substances ~~have been determined~~ or will be determined ~~as of~~ * the end of the study.

AT Analytical 2/27/87

The above information and documentation are located in the sponsor's records.

~~Sponsor or~~ Sponsor Representative	Date
[signature]	4/27/87

Original Characterization can be found in Exp. # 0387MB0057

* = Form change

PAIN...

Form 19793-A PWO

APPENDIX IV

QUALITY ASSURANCE STATEMENT

Acute Toxicology Laboratory Studies

Study No.: 0387EG0059

This short term study was audited by Compliance Audit and the final report examined against the raw data on December 18, 1987. The results of the audit were reported to the study director and to management on December 18, 1987.

In addition to the data audit, different significant phases for studies underway in the Acute Toxicology Laboratory are inspected on a recurring cycle, and the facilities are examined by Compliance Audit on a yearly schedule.

D. M. Marbaet

Compliance Audit

12-19-87

Date

Pathology and Toxicology
Riker Laboratories, Inc.

Building 270-3S-05, 3M Center
St. Paul, Minnesota 55144-1000

COMPANY CONFIDENTIAL

3M

Repeat Skin Irritation Test

with Hydroxypropylmethylcellulose Gel Containing

50% w/w Pyridostigmine Bromide, Lot FN4588

in Albino Rabbits

Riker Experiment No: 0387EB0073

Conducted At: Pathology and Toxicology
Riker Laboratories, Inc.

Dates Conducted: March 17, 1987 to March 24, 1987

Conducted By: Gene L. Harris 4/22/87

G. L. Harris, B.S. Date
Advanced Toxicologist
Study Director and Acute Toxicity
Study Coordinator

Reviewed By: J L Allen 4-22-87

J.L. Allen, Ph.D. Date
Diplomate, A.B.T.
Research Toxicologist

dc: R.T. Catherall
M.W. Downing
N.M. Marecki
M.J. Westfall (2)
Tech. Doc. Center
Path/Tox Files

Summary

The results of the cumulative skin irritation test conducted from March 17, 1987 to March 24, 1987 at Riker Laboratories, Inc., St. Paul, Minnesota indicate that hydroxypropylmethylcellulose gel with 50% pyridostigmine bromide produced slight cumulative skin irritation in female albino rabbits when administered to the same test site daily for 7 consecutive days. The initial mean irritation score of 1.0 for erythema and 0.7 for edema was produced after 1 application of the test material and diarrhea and tremors were noted in one rabbit at 1 hour after the initial dose. The mean irritation scores increased to a maximum of 1.7 for erythema and 1.0 for edema by day 3. The mean irritation decreased to a score of 1.5 for erythema and 0.3 for edema at the final observation for this study on day 7.

Introduction

The objective of this study was to determine the cumulative skin irritation potential of hydroxypropylmethylcelluose gel with 50% pyridostigmine bromide to the skin of female albino rabbits. This study was conducted in accordance with the Food and Drug Administration's Good Laboratory Practice Regulation of 1978. The raw data generated by the Study Director and the final report are stored in the conducting laboratory's archives.

Animals and Husbandry

Female young New Zealand White Rabbits[a] were used in the evaluation of the cumulative skin irritating properties of the test article. The rabbits were individually housed[b] in stainless steel cages, and food[c] and water were available ad libitum. All rabbits were individually identified with ear tags and considered to be in good health at study initiation. The rabbits were housed in a temperature and humidity controlled room. Room lighting was on a 12/12 hour light/dark cycle that was automatically timed.

Method and Results

The test procedure was modeled after that of Draize et al[d]. Prior to the initiation of the study, the hair was clipped from the back and flanks of each rabbit and one test site was selected lateral to the midline of the back.

The test article was applied to the skin at the same test site on seven consecutive days. The test article (0.1 ml for dose 1 and 0.05 ml for the remaining 6 doses), was applied to the test site on each rabbit and covered with gauze. The trunk of each animal was then wrapped with impervious plastic sheeting[e] which occluded the test article during the 23 hour contact period.

[a] Hazleton Dutchland, Inc., Denver, PA
[b] Animals were housed in accordance with recommendations contained in DHEW Publication No. 85-23 (NIH): Revised 1985 "Guide For the Care and Use of Laboratory Animals."
[c] Purina Lab Rabbit Chow and rabbits may be offered Alfalfa Cubes for additional roughage.
[d] Draize: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965).
[e] 10 x 12 x .002 Extra Clear polyethylene sleeves, PPC Industries, Inc., Wheeling, IL

At the end of the exposure period the plastic wrappings and all residual test article were removed by washing with water. One hour after removal of the test article the intact test site was examined and scored for erythema and edema on a graded scale of 0 - 4.

The irritation produced was evaluated by means of the daily average scores for erythema and edema of the intact test site one hour post removal of the test article. The scoring criteria for erythema and edema are shown below.

Scoring Criteria for Skin Reactions

Reaction	Description	Score
Erythema	Barely perceptible (Edges of area not defined)	1
	Pale red in color and area definable	2
	Definite red in color and area well defined.	3
	Beet or crimson red in color	4
Edema	Barely perceptible (Edges of area not defined)	1
	Area definable but not raised more than 1 mm.	2
	Area well defined and raised approximately 1 mm.	3
	Area raised more than 1 mm.	4
	Maximum Primary Irritation Score =	8

The results are presented in Table 1. The protocol, principal personnel involved in the study, composition characteristics and Quality Assurance statement are contained in Appendices I - IV.

Table 1

Repeat Skin Irritation Test - Albino Rabbits

with Hydroxypropylmethylcellulose Gel with

50% w/w Pyridostigmine Bromide

1 Hour
Irritation Scores for Intact
Skin Sites after Removal:

Animal Number	Day 1 ER.	Day 1 ED.	Day 2 ER.	Day 2 ED.	Day 3 ER.	Day 3 ED.	Day 4 ER.	Day 4 ED.	Day 5 ER.	Day 5 ED.
7B295	2	1	2	2	3	2	3	1	2	1
7B298	1	1	2	1	2	1	2	1	2	0
7B301*	2	1	2	1	2	1	2	1	2	0
7B304	0	0	0	0	0	0	0	0	1	0
7B246	1	1	1	1	1	1	1	1	1	1
7B272	0	0	1	0	2	1	2	1	2	1
Mean	1.0	0.7	1.3	0.8	1.7	1.0	1.7	0.8	1.7	0.5

Animal Number	Day 6 ER.	Day 6 ED.	Day 7 ER.	Day 7 ED.
7B295	2	1	2	1
7B298	2	0	1	0
7B301	2	0	2	0
7B304	1	0	1	0
7B246	1	0	1	0
7B272	2	1	2	1
Mean	1.7	0.3	1.5	0.3

Key: ER. = Erythema
ED. = Edema
E = Epithelial Stripping
* = Diarrhea and tremors were noted in this rabbit at one hour after dose administration of dose 1.

RIKER Experiment No.: 0387EB0073

~~771~~

APPENDIX I

PROTOCOL

5.

GLP STUDY

TEST: Skin Irritation Test (Repeat Application)

SPONSOR: 3M RIKER Division

CONDUCTED BY: **Safety Evaluation Laboratory, Riker Laboratories, Inc., St. Paul, Minnesota**

TEST ARTICLE: Hydroxypropylmethylcellulose gel containing 50% w/w Pyridostigmine Bromide, Lot FN4588

PROPOSED STARTING/COMPLETION DATE OF TEST: 3/87 - 7/87

TEST SYSTEM: Female New Zealand White Albino Rabbits

SOURCE: Hazleton-Dutchland, Denver, PA

OBJECTIVE: To assess the irritation potential of the test article to the skin of female animals after repeat contact. Rabbits were selected as the test system due to their historical use, sensitivity to irritants, ease of handling and general availability.

METHOD: The animals will be housed in standard wire-mesh cages in temperature and humidity controlled rooms with food[a] and water offered ad libitum. Six animals will be used for this test. Each animal will be assigned a numbered ear tag, which will correspond to a card affixed to the outside of the cage. The test article will be applied to the skin at the same test site on four to seven consecutive days. Prior to the application of the test article, the hair will be clipped from the back and flanks of each animal and one test site selected lateral to the midline of the back. The test site will remain intact. The test article 0.1 ml will be applied to the intact site on each animal, covered with gauze and secured with gauze. The trunk of each animal will then be wrapped with impervious plastic sheeting which will occlude the test article during the 23 hour exposure period. Approximately one hour after removal of the test article, the intact test site will be examined and scored for erythema and edema on a graded scale of 0 to 4[b]. The irritation produced will be evaluated by meaning the scores for erythema and edema of the intact test site one hour post removal of the test article for each application. These values will be assessed for potential cumulative irritation. The raw data generated by the study director and the final report will be stored in the Riker Laboratories' Archive, St. Paul, Minnesota.

[a] Purina Rabbit Chow, Ralston Purina Co., St. Louis, Missouri

[b] Draize: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965) Published by the Editorial Committee of the Association of Food and Drug Officials of the United States.

Marie Westfall 3/11/87
Sponsor Date

Gene L. Harris 3/16/87
Study Director Date

Riker Experiment No. 0387EB0073

Appendix I (concluded)
Deviations and/or Amendments to Protocol

1. A dose of 0.05 ml per animal will be administered starting with dose #2. Reason for change: to avoid mortality of the animals as a result of the test material dose volume.

Gene Harris 3/18/87
Study Director Date

2.

Study Director Date

3.

Study Director Date

4.

Study Director Date

APPENDIX II

Principal Participating Personnel Involved in the Study

Name	Function
G. L. Harris, B.S.	Advanced Toxicologist Study Director
G. E. Hart	Master Laboratory Technician Acute Toxicology
L. O. Wiseth	Sr. Laboratory Technician Acute Toxicology
J. A. Eads	Jr. Laboratory Technician Acute Toxicology
J. L. Allen, Ph.D.	Research Toxicologist Acute Toxicology
G. C. Pecore	Supervisor Animal Laboratory

APPENDIX III

Test and/or Control Article Characterization

for

Hydroxypropylmethylcellulose gel containing 50% w/w pyridostigmine Bromide (FN 4588)

1. The identity strength, uniformity, composition, purity or other pertinent characterizations of the test and/or substances have been determined and documented as of RFA 14203 - 2/27/87.

2. The method of synthesis or origin of the test and control substances, including their amount and the method of bioassay (if applicable) is documented.

   ☐ Yes ☐ No Amit a Tita 2/27/87

3. The stability of the test and/or control substances ~~have been determined~~ ~~or~~ will be determined ~~as of~~* At end of study.
   AT Amit a Tita (2/27/87)

The above information and documentation are located in the sponsor's records.

~~Sponsor or~~ Sponsor Representative	Date
Amit a Tita	2/27/87

* = Form CHANGE

PATH...

APPENDIX IV

QUALITY ASSURANCE STATEMENT

Acute Toxicology Laboratory Studies

Study No.: 0387EB0073

This short term study was audited by Compliance Audit and the final report examined against the raw data on April 21, 1987. The results of the audit were reported to the study director and to management on April 21, 1987.

In addition to the data audit, different significant phases for studies underway in the Acute Toxicology Laboratory are inspected on a recurring cycle, and the facilities are examined by Compliance Audit on a three month schedule.

D. M. Markoc, Jr.
Compliance Audit

4-21-87
Date

Riker Pathology and Toxicology Department Services Request

(Pathology and Toxicology Department Use Only)

Experiment Number 3387530073 776

To:	From:	Date
GENE HARRIS	[illegible]	3/11/87
3M Division	**Address**	**Phone**	**Riker Project No.**
Riker	270-45-02	6-1367	9C21000803

Test Article Information

Sample Name and/or I.D. No.	Lot Number
(3-26741) Hydroxypropium [illegible]	FA14587

Test Article Storage Conditions: ☒ Room Temp. ☐ Refrigerate ☐ Other _____

Proposed End Use of Product: Transdermal Patch

The following service is requested on the test article listed above:

Irritation Studies

- ☐ Primary Skin Irritation (Rabbit)
- ☒ Four Day Repeat Skin Irritation (Rabbit)
- ☐ Primary Eye Irritation (Rabbit)
- ☐ Mucous Membrane Irritation (Hamster)
- ☐ Intracutaneous Irritation (Rabbit)

(Circle extracting mediums required)

☐ Saline ☐ 1:20 Ethanol : Saline
☐ Cottonseed oil ☐ Peg 400

Sensitization Studies

- ☐ Magnusson - Kligman Maximization (Guinea Pig)
- ☐ Buehler Sensitization (Guinea Pig)

Acute Toxicity Studies

- ☐ Acute Oral Limit Test (1 level in rats)
- ☐ Acute Oral LD_{50} (Rat)
- ☐ Acute Dermal Limit Test (1 level in rabbits)
- ☐ Acute Dermal LD_{50} (Rabbits)
- ☐ Acute I.V. LD_{50} (List species _____)
- ☐ Acute I.P. LD_{50} (List species _____)
- ☐ Acute Systemic Toxicity (USP-Mice)

(Circle extracting mediums required)

☐ Saline ☐ 1:20 Ethanol : Saline
☐ Cottonseed oil ☐ Peg 400

Invitro Studies

- ☐ Agar Overlay
- ☐ Cell Growth Inhibition
- ☐ Direct Cell Contact

Special Services Requested

Regulatory Compliance

☒ All studies are to be conducted following the Good Laboratories Practices Act in accordance with ☒ FDA ☐ EPA (FIFRA) ☐ EPA (TSCA) ☐ OECD Governmental Requirements.

☐ All studies are for research and development and are not intended to support a governmental submission or marketing permit.

☐ Other: Explain _____

Authorized By ► Marie Wolf Date 3/11/87

Comments

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PATHOLOGY AND TOXICOLOGY

Form 21773 - E - PWO White - Raw Data File Canary - Requestor 3M "Action" 200 Paper

RIKER Experiment No.: 0387EB0073

PROTOCOL

TEST: Skin Irritation Test (Repeat Application) G.P. STUDY 777

SPONSOR: 3M RIKER Division

CONDUCTED BY: **Safety Evaluation Laboratory, Riker Laboratories, Inc., St. Paul, Minnesota**

TEST ARTICLE: [illegible]

PROPOSED STARTING/COMPLETION DATE OF TEST: 3/87 - 7/87

TEST SYSTEM: Female New Zealand White Albino Rabbits

SOURCE: Hazleton-Dutchland, Denver, PA

OBJECTIVE: To assess the irritation potential of the test article to the skin of female animals after repeat contact. Rabbits were selected as the test system due to their historical use, sensitivity to irritants, ease of handling and general availability.

METHOD: The animals will be housed in standard wire-mesh cages in temperature and humidity controlled rooms with food[a] and water offered **ad libitum**. Six animals will be used for this test. Each animal will be assigned a numbered ear tag, which will correspond to a card affixed to the outside of the cage. The test article will be applied to the skin at the same test site on four to seven consecutive days. Prior to the application of the test article, the hair will be clipped from the back and flanks of each animal and one test site selected lateral to the midline of the back. The test site will remain intact. The test article 0.1 mL will be applied to the intact site on each animal, covered with gauze and secured with gauze. The trunk of each animal will then be wrapped with impervious plastic sheeting which will occlude the test article during the 23 hour exposure period. Approximately one hour after removal of the test article, the intact test site will be examined and scored for erythema and edema on a graded scale of 0 to 4[b]. The irritation produced will be evaluated by meaning the scores for erythema and edema of the intact test site one hour post removal of the test article for each application. These values will be assessed for potential cumulative irritation. The raw data generated by the study director and the final report will be stored in the Riker Laboratories' Archive, St. Paul, Minnesota.

[a] Purina Rabbit Chow, Ralston Purina Co., St. Louis, Missouri

[b] Draize: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965) Published by the Editorial Committee of the Association of Food and Drug Officials of the United States.

Marie Wolfe 3/11/87

Sponsor Date

Study Director Date

Pathology and Toxicology
Riker Laboratories, Inc.

Building 270-3S-05, 3M Center
St. Paul, Minnesota 55144-1000

COMPANY CONFIDENTIAL

3M

Repeat Skin Irritation Test

with Hydroxyproplymethylcellulose Gel Containing

30% Pyridostigmine Bromide and 0.21% Docusate Sodium, Lot FN4589

in Albino Rabbits

Riker Experiment No: 0387EB0074

Conducted At: Pathology and Toxicology
Riker Laboratories, Inc.

Dates Conducted: March 17, 1987 to March 24, 1987

Conducted By: Gina L. Harris 4/20/87
G. L. Harris, B.S. Date
Advanced Toxicologist
Study Director and Acute Toxicity
Study Coordinator

Reviewed By: J Allen 4-22-87
J. L. Allen, Ph.D. Date
Diplomate, A.B.T.
Research Toxicologist

dc: J.L. Allen
R.T. Catherall
M.W. Downing
N.M. Marecki
M.J. Westfall (2)
Tech. Doc. Center
Path/Tox File

Summary

The results of the cumulative skin irritation test conducted from March 17, 1987 to March 24, 1987 at Riker Laboratories, Inc., St. Paul, Minnesota indicate that hydroxypropylmethylcellulose gel with 30% pyridostigmine bromide and 0.21% docusate sodium produced moderate cumulative skin irritation in female rabbits when administered to the same test site daily for 7 consecutive days. The initial mean irritation score of 0.8 for erythema and 0.2 for edema was produced after one application of the test material. The mean irritation scores increased to a maximum of 2.0 for erythema on day 5 and 1.4 for edema on day 7. The mean irritation score for erythema decreased to 1.8 by the final observation on day 7. One rabbit was found dead during the study at one hour after dose administration of dose seven. Salivation and tremors were noted in the rabbit just prior to death.

Introduction

The objective of this study was to determine the cumulative skin irritation potential of hydroxypropylmethylcellulose gel with 30% pyridostigmine bromide and 0.21% docusate sodium to the skin of female albino rabbits. This study was conducted in accordance with the Food and Drug Administration's Good Laboratory Practice Regulation of 1978. The raw data generated by the Study Director and the final report are stored in the conducting laboratory's archives.

Animals and Husbandry

Female young New Zealand White Rabbits[a] were used in the evaluation of the cumulative skin irritating properties of the test article. The rabbits were individually housed[b] in stainless steel cages, and food[c] and water were available ad libitum. All rabbits were individually identified with ear tags and considered to be in good health at study initiation. The rabbits were housed in a temperature and humidity controlled room. Room lighting was on a 12/12 hour light/dark cycle that was automatically timed.

Method and Results

The test procedure was modeled after that of Draize et al[d]. Prior to the initiation of the study, the hair was clipped from the back and flanks of each rabbit and one intact site was selected lateral to the midline of the back.

The test article was applied to the skin at the same test site on seven consecutive days. The test article (0.1 ml for dose 1 and 0.05 ml for the remaining 6 doses), was applied to the test site on each rabbit and covered with gauze. The trunk of each animal was then wrapped with impervious plastic sheeting[e] which occluded the test article during the 23 hour contact period.

[a] Hazleton Dutchland, Inc., Denver, PA
[b] Animals were housed in accordance with recommendations contained in DHEW Publication No. 85-23 (NIH): Revised 1985 "Guide For the Care and Use of Laboratory Animals."
[c] Purina Lab Rabbit Chow and rabbits may be offered Alfalfa Cubes for additional roughage.
[d] Draize: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965).
[e] 10 x 12 x .002 Extra Clear polyethylene sleeves, PPC Industries, Inc., Wheeling, IL

At the end of the exposure period the plastic wrappings and all residual test article were removed by washing with water. One hour after removal of the test article, the intact test site was examined and scored for erythema and edema on a graded scale of 0 - 4.

The irritation produced was evaluated by means of the daily average scores for erythema and edema of the intact test site one hour post removal of the test article. The scoring criteria for erythema and edema are shown below.

Scoring Criteria for Skin Reactions

Reaction	Description	Score
Erythema	Barely perceptible (Edges of area not defined)	1
	Pale red in color and area definable	2
	Definite red in color and area well defined.	3
	Beet or crimson red in color	4
Edema	Barely perceptible (Edges of area not defined)	1
	Area definable but not raised more than 1 mm.	2
	Area well defined and raised approximately 1 mm.	3
	Area raised more than 1 mm.	4
	Maximum Primary Irritation Score =	8

The results are presented in Table 1. The protocol, principal personnel involved in the study, composition characteristics and Quality Assurance statement are contained in Appendices I - IV.

Table 1

Repeat Skin Irritation Test - Albino Rabbits

with Hydroxypropylmethylcellulose Gel With

30% Pyridostigmine Bromide + 0.21% Docusate Sodium

1 Hour
Irritation Scores for Intact
Skin Sites after Removal:

Animal Number	Day 1		Day 2		Day 3		Day 4		Day 5	
	ER.	ED.	ER.	ED.	ER.	ED.	ER.	ED.	ER.	ED.
7B302	1	0	1	0	1	0	1	0	2	0
7B305	1	1	2	1	2	1	2	1	2	1
7B297	1	0	1	1	2	1	2	1	2	2
7B271	0	0	1	0	2	1	2	2	2	1
7B292	0	0	0	0	1	1	1	1	1	0
7B270	2	0	2	1	2	2	2	2	3	2
Mean	0.8	0.2	1.2	0.5	1.7	1.0	1.7	1.2	2.0	1.0

Animal Number	Day 6		Day 7	
	ER.	ED.	ER.	ED.
7B302	2	1	2	1
7B305	2	1	X	-
7B297	2	2	2	2
7B271	2	1	2	1
7B292	1	1	1	1
7B270	3	2	2	2
Mean	2.0	1.3	1.8	1.4

Key: ER. = Erythema
ED. = Edema
E = Epithelial Stripping
X = Rabbit 7B305 was found dead at 1 hour post dose administration of dose seven with salivation and tremors noted in this rabbit just prior to death.

RIKER Experiment No.: 0387EB0074

APPENDIX I

PROTOCOL

783

5.

GLP STUDY

TEST: Skin Irritation Test (Repeat Application)

SPONSOR: 3M RIKER Division

CONDUCTED BY: **Safety Evaluation Laboratory, Riker Laboratories, Inc., St. Paul, Minnesota**

TEST ARTICLE: Hydroxypropylmethylcellulose gel containing 30% Pyridostigmine Bromide and 0.2% Docusate Sodium, Lot FN 4589

PROPOSED STARTING/COMPLETION DATE OF TEST: 3/87 - 7/87

TEST SYSTEM: Female New Zealand White Albino Rabbits

SOURCE: Hazleton-Dutchland, Denver, PA

OBJECTIVE: To assess the irritation potential of the test article to the skin of female animals after repeat contact. Rabbits were selected as the test system due to their historical use, sensitivity to irritants, ease of handling and general availability.

METHOD: The animals will be housed in standard wire-mesh cages in temperature and humidity controlled rooms with food[a] and water offered ad libitum. Six animals will be used for this test. Each animal will be assigned a numbered ear tag, which will correspond to a card affixed to the outside of the cage. The test article will be applied to the skin at the same test site on four to seven consecutive days. Prior to the application of the test article, the hair will be clipped from the back and flanks of each animal and one test site selected lateral to the midline of the back. The test site will remain intact. The test article 0.1 mL will be applied to the intact site on each animal, covered with gauze and secured with gauze. The trunk of each animal will then be wrapped with impervious plastic sheeting which will occlude the test article during the 23 hour exposure period. Approximately one hour after removal of the test article, the intact test site will be examined and scored for erythema and edema on a graded scale of 0 to 4[b]. The irritation produced will be evaluated by meaning the scores for erythema and edema of the intact test site one hour post removal of the test article for each application. These values will be assessed for potential cumulative irritation. The raw data generated by the study director and the final report will be stored in the Riker Laboratories' Archive, St. Paul, Minnesota.

[a] Purina Rabbit Chow, Ralston Purina Co., St. Louis, Missouri.

[b] Draize: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965) Published by the Editorial Committee of the Association of Food and Drug Officials of the United States.

Sponsor 3/11/87 Date

Study Director 3/16/87 Date

Riker Experiment No. 0387EB0074

Appendix I (concluded)
Deviations and/or Amendments to Protocol

1. A dose of 0.05 ml per animal will be administered starting with dose #2. Reason for change: to avoid mortality of the animals as a result of the test material dose volume.

Gene Harris 3/18/87
Study Director Date

2.

Study Director Date

3.

Study Director Date

4.

Study Director Date

APPENDIX II

Principal Participating Personnel Involved in the Study

Name	Function
G. L. Harris, B.S.	Advanced Toxicologist Study Director
G. E. Hart	Master Laboratory Technician Acute Toxicology
L. O. Wiseth	Sr. Laboratory Technician Acute Toxicology
J. A. Eads	Jr. Laboratory Technician Acute Toxicology
J. L. Allen, Ph.D.	Research Toxicologist Acute Toxicology
G. C. Pecore	Supervisor Animal Laboratory

APPENDIX III

Test and/or Control Article Characterization

for

Hydroxypropylmethylcellulose gel containing 30% pyridostigmine Bromide and 0.2% Docusate Sodium, (FN 4589)

1. The identity strength, uniformity, composition, purity or other pertinent characterizations of the test and/or substances have been determined and documented as of RIA 14201 – 2/27/87.

2. The method of synthesis or origin of the test and control substances, including their amount and the method of bioassay (if applicable) is documented.

☐ Yes ☐ No Anita Toth 2/27/87

3. The stability of the test and/or control substances* ~~have been determined or~~ will be determined ~~as of~~ AT the end of the study.

Anita Toth 2/27/87

The above information and documentation are located in the sponsor's records.

~~Sponsor or~~ Sponsor Representative	Date
Anita Toth	2/27/87

* = Form change

APPENDIX IV

QUALITY ASSURANCE STATEMENT

Acute Toxicology Laboratory Studies

Study No.: 0387EB0074

This short term study was audited by Compliance Audit and the final report examined against the raw data on April 21, 1987. The results of the audit were reported to the study director and to management on April 21, 1987.

In addition to the data audit, different significant phases for studies underway in the Acute Toxicology Laboratory are inspected on a recurring cycle, and the facilities are examined by Compliance Audit on a three month schedule.

D. M. Marboe

Compliance Audit

4-21-87

Date

Pathology and Toxicology
Riker Laboratories, Inc.

Building 270-3S-05, 3M Center
St. Paul, Minnesota 55144-1000

COMPANY CONFIDENTIAL

3M

Repeat Skin Irritation Test with Hydroxypropylmethylcellulose Gel Containing 30% Pyridostigmine Bromide and 0.198% Sodium Lauryl Sulfate, Lot FN4590 in Albino Rabbits

Riker Experiment No: 0387EB0075

Conducted At: Pathology and Toxicology
Riker Laboratories, Inc.

Dates Conducted: March 17, 1987 to March 24, 1987

Conducted By: Gene L. Harris 4/20/87
G. L. Harris, B.S. Date
Advanced Toxicologist
Study Director and Acute Toxicity
Study Coordinator

Reviewed By: J. Allen 4-22-87
J. L. Allen, Ph.D. Date
Diplomate, A.B.T.
Research Toxicologist

dc: J.L Allen
R.T. Catherall
M.W. Downing
N.M. Marecki
M.J. Westfall (2)
Tech. Doc. Center
Path/Tox File

Summary

The results of the cumulative skin irritation test conducted from March 17, 1987 to March 24, 1987 at Riker Laboratories, Inc., St. Paul, Minnesota indicate that hydroxypropylmethylcellulose gel with 30% pyridostigmine bromide and 0.198% sodium lauryl sulfate produced moderate cumulative skin irritation in female albino rabbits when administered to the same test site daily for seven consecutive days. The initial mean irritation score of 1.0 for erythema and 0.2 for edema was produced after 1 application of the test material. The mean irritation scores increased to a maximum of 2.7 for erythema and 2.0 for edema by Day 6. The mean irritation score was 2.3 for erythema and 2.0 for edema at the final observation on Day 7. Three of the animals on this study were found dead. All deaths occurred at approximately 1 hour after dose administration with 1 animal each dying on Day 2, Day 3 and Day 4. Diarrhea and tremors were noted in all animals found dead just prior to death. Diarrhea and tremors were also noted in 1/6 animals at 1 hour after administration of the initial dose.

Introduction

The objective of this study was to determine the cumulative skin irritation potential of hydroxypropylmethylcellulose gel with 30% pyridostigmine bromide and 0.198% sodium lauryl sulfate to the skin of female albino rabbits. This study was conducted in accordance with the Food and Drug Administration's Good Laboratory Practice Regulation of 1978. The raw data generated by the Study Director and the final report are stored in the conducting laboratory's archives.

Animals and Husbandry

Female young New Zealand White Rabbits[a] were used in the evaluation of the cumulative skin irritating properties of the test article. The rabbits were individually housed[b] in stainless steel cages, and food[c] and water were available ad libitum. All rabbits were individually identified with ear tags and considered to be in good health at study initiation. The rabbits were housed in a temperature and humidity controlled room. Room lighting was on a 12/12 hour light/dark cycle that was automatically timed.

Method and Results

The test procedure was modeled after that of Draize et al[d]. Prior to the initiation of the study, the hair was clipped from the back and flanks of each rabbit and one intact site was selected lateral to the midline of the back.

The test article was applied to the skin at the same test site on seven consecutive days. The test article (0.1 ml for dose 1 and 0.05 ml for the remaining 6 doses), was applied to the test site on each rabbit and covered with gauze. The trunk of each animal was then wrapped with impervious plastic sheeting[e] which occluded the test article during the 23 hour contact period.

[a] Hazleton Dutchland, Inc., Denver, PA
[b] Animals were housed in accordance with recommendations contained in DHEW Publication No. 85-23 (NIH): Revised 1985 "Guide For the Care and Use of Laboratory Animals."
[c] Purina Lab Rabbit Chow and rabbits may be offered Alfalfa Cubes for additional roughage.
[d] Draize: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965).
[e] 10 x 12 x .002 Extra Clear polyethylene sleeves, PPC Industries, Inc., Wheeling, IL

At the end of the exposure period the plastic wrappings and all residual test article were removed by washing with water. One hour after removal of the test article, the intact test site was examined and scored for erythema and edema on a graded scale of 0 - 4.

The irritation produced was evaluated by means of the daily average scores for erythema and edema of the intact test site one hour post removal of the test article. The scoring criteria for erythema and edema are shown below.

Scoring Criteria for Skin Reactions

Reaction	Description	Score
Erythema	Barely perceptible (Edges of area not defined)	1
	Pale red in color and area definable	2
	Definite red in color and area well defined.	3
	Beet or crimson red in color	4
Edema	Barely perceptible (Edges of area not defined)	1
	Area definable but not raised more than 1 mm.	2
	Area well defined and raised approximately 1 mm.	3
	Area raised more than 1 mm.	4
	Maximum Primary Irritation Score =	8

The results are presented in Table 1. The protocol, principal personnel involved in the study, composition characteristics and Quality Assurance statement are contained in Appendices I - IV.

Table 1

Repeat Skin Irritation Test - Albino Rabbits

with Hydroxypropylmethylcellulose Gel With

30% Pyridostigmine Bromide + 0.198% Sodium Lauryl Sulfate

1 Hour
Irritation Scores for Intact
Skin Sites after Removal:

Animal Number	Day 1 ER.	Day 1 ED.	Day 2 ER.	Day 2 ED.	Day 3 ER.	Day 3 ED.	Day 4 ER.	Day 4 ED.	Day 5 ER.	Day 5 ED.
7B318@	1	0	X	-	-	-	-	-	-	-
7B365	1	0	1	0	1	1	x	-	-	-
7B357	1	1	1	1	1	1	1	1	2	1
7B360	1	0	2	0	2	1	2	2	2	1
7B363	1	0	2	1	X	-	-	-	-	-
7B366	1	0	2	0	2	1	2	1	2	1
Mean	1.0	0.2	1.6	0.4	1.5	1.0	1.7	1.3	2.0	1.0

Animal Number	Day 6 ER.	Day 6 ED.	Day 7 ER.	Day 7 ED.
7B318	-	-	-	-
7B365	-	-	-	-
7B357	2	2	2	2
7B360	3	2	2	2
7B363	-	-	-	-
7B366	3	2	3	2
Mean	2.7	2.0	2.3	2.0

Key: ER. = Erythema
ED. = Edema
E = Epithelial Stripping
@ = Tremors and diarrhea were noted 1 hour after dose administration of dose 1.
X = This animal was found dead 1 hour after dose administration with diarrhea and tremors noted prior to death.

RIKER Experiment No.: 0387EB0075

APPENDIX I

PROTOCOL

792

5.

GLP STUDY

TEST: Skin Irritation Test (Repeat Application)

SPONSOR: 3M RIKER Division

CONDUCTED BY: **Safety Evaluation Laboratory, Riker Laboratories, Inc., St. Paul, Minnesota**

TEST ARTICLE: Hydroxy propyl methyl cellulose gel containing 30% pyridostigmine Bromide and 0.198% Sodium lauryl Sulfate, Lot FN4590

PROPOSED STARTING/COMPLETION DATE OF TEST: 3/87 - 7/87

TEST SYSTEM: Female New Zealand White Albino Rabbits

SOURCE: Hazleton-Dutchland, Denver, PA

OBJECTIVE: To assess the irritation potential of the test article to the skin of female animals after repeat contact. Rabbits were selected as the test system due to their historical use, sensitivity to irritants, ease of handling and general availability.

METHOD: The animals will be housed in standard wire-mesh cages in temperature and humidity controlled rooms with food[a] and water offered ad libitum. Six animals will be used for this test. Each animal will be assigned a numbered ear tag, which will correspond to a card affixed to the outside of the cage. The test article will be applied to the skin at the same test site on four to seven consecutive days. Prior to the application of the test article, the hair will be clipped from the back and flanks of each animal and one test site selected lateral to the midline of the back. The test site will remain intact. The test article 0.1 ml will be applied to the intact site on each animal, covered with gauze and secured with gauze. The trunk of each animal will then be wrapped with impervious plastic sheeting which will occlude the test article during the 23 hour exposure period. Approximately one hour after removal of the test article, the intact test site will be examined and scored for erythema and edema on a graded scale of 0 to 4[b]. The irritation produced will be evaluated by meaning the scores for erythema and edema of the intact test site one hour post removal of the test article for each application. These values will be assessed for potential cumulative irritation. The raw data generated by the study director and the final report will be stored in the Riker Laboratories' Archive, St. Paul, Minnesota.

[a] Purina Rabbit Chow, Ralston Purina Co., St. Louis, Missouri.

[b] Draize: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965) Published by the Editorial Committee of the Association of Food and Drug Officials of the United States.

Marie Wolfe 3/11/87
Sponsor Date

Gene L. Harris 3/16/87
Study Director Date

Riker Experiment No. 0387EB0075

Appendix I (concluded)
Deviations and/or Amendments to Protocol

1. A dose of 0.05 ml per animal will be administered starting with dose #2. Reason for change: to avoid mortality of the animals as a result of the test material dose volume.

Gene Harris 3/18/87
Study Director Date

2. _____

Study Director Date

3. _____

Study Director Date

4. _____

Study Director Date

APPENDIX II

Principal Participating Personnel Involved in the Study

Name	Function
G. L. Harris, B.S.	Advanced Toxicologist Study Director
G. E. Hart	Master Laboratory Technician Acute Toxicology
L. O. Wiseth	Sr. Laboratory Technician Acute Toxicology
J. A. Eads	Jr. Laboratory Technician Acute Toxicology
J. L. Allen, Ph.D.	Research Toxicologist Acute Toxicology
G. C. Peoore	Supervisor Animal Laboratory

APPENDIX III

Test and/or Control Article Characterization

for

Hydroxypropyl methylcellulose gel containing 30% pyridostigmine Bromide and 0.198% Sodium Lauryl Sulfate, (FN 4590)

1. The identity strength, uniformity, composition, purity or other pertinent characterizations of the test and/or substances have been determined and documented as of RFA 14202-2/27/87.

2. The method of synthesis or origin of the test and control substances, including their amount and the method of bioassay (if applicable) is documented.

☐ Yes ☐ No Anita Tilton 2/27/87

3. The stability of the test and/or control substances ~~have been determined~~ * ~~or~~ will be determined ~~as of~~ AT the end of the Study.

Anita Tilton 2/27/87

The above information and documentation are located in the sponsor's records.

~~Sponsor or~~ Sponsor Representative	Date
Anita Tilton	2/27/87

* = Form change

APPENDIX IV

QUALITY ASSURANCE STATEMENT

Acute Toxicology Laboratory Studies

Study No.: 0387EB0075

This short term study was audited by Compliance Audit and the final report examined against the raw data on April 21, 1987. The results of the audit were reported to the study director and to management on April 21, 1987.

In addition to the data audit, different significant phases for studies underway in the Acute Toxicology Laboratory are inspected on a recurring cycle, and the facilities are examined by Compliance Audit on a three month schedule.

D. M. Warbood

Compliance Audit

4-21-87

Date

Pathology and Toxicology
Riker Laboratories, Inc.

Building 270-3S-05, 3M Center
St. Paul, Minnesota 55144-1000

COMPANY CONFIDENTIAL

3M

Repeat Skin Irritation Test

with Hydroxyproplymethylcellulose Gel Containing

30% Pyridostigmine Bromide and 0.21% Docusate Sodium, Lot FN4589

in Albino Rabbits

Riker Experiment No:	0387EB0074
Conducted At:	Pathology and Toxicology Riker Laboratories, Inc.
Dates Conducted:	March 17, 1987 to March 24, 1987
Conducted By:	Gina L. Harris 4/20/87 G. L. Harris, B.S. Date Advanced Toxicologist Study Director and Acute Toxicity Study Coordinator
Reviewed By:	J Allen 4-22-87 J. L. Allen, Ph.D. Date Diplomate, A.B.T. Research Toxicologist

dc: J.L. Allen
R.T. Catherall
M.W. Downing
N.M. Marecki
M.J. Westfall (2)
Tech. Doc. Center
Path/Tox File

Summary

The results of the cumulative skin irritation test conducted from March 17, 1987 to March 24, 1987 at Riker Laboratories, Inc., St. Paul, Minnesota indicate that hydroxypropylmethylcellulose gel with 30% pyridostigmine bromide and 0.21% docusate sodium produced moderate cumulative skin irritation in female rabbits when administered to the same test site daily for 7 consecutive days. The initial mean irritation score of 0.8 for erythema and 0.2 for edema was produced after one application of the test material. The mean irritation scores increased to a maximum of 2.0 for erythema on day 5 and 1.4 for edema on day 7. The mean irritation score for erythema decreased to 1.8 by the final observation on day 7. One rabbit was found dead during the study at one hour after dose administration of dose seven. Salivation and tremors were noted in the rabbit just prior to death.

Introduction

The objective of this study was to determine the cumulative skin irritation potential of hydroxypropylmethylcellulose gel with 30% pyridostigmine bromide and 0.21% docusate sodium to the skin of female albino rabbits. This study was conducted in accordance with the Food and Drug Administration's Good Laboratory Practice Regulation of 1978. The raw data generated by the Study Director and the final report are stored in the conducting laboratory's archives.

Animals and Husbandry

Female young New Zealand White Rabbits[a] were used in the evaluation of the cumulative skin irritating properties of the test article. The rabbits were individually housed[b] in stainless steel cages, and food[c] and water were available ad libitum. All rabbits were individually identified with ear tags and considered to be in good health at study initiation. The rabbits were housed in a temperature and humidity controlled room. Room lighting was on a 12/12 hour light/dark cycle that was automatically timed.

Method and Results

The test procedure was modeled after that of Draize et al[d]. Prior to the initiation of the study, the hair was clipped from the back and flanks of each rabbit and one intact site was selected lateral to the midline of the back.

The test article was applied to the skin at the same test site on seven consecutive days. The test article (0.1 ml for dose 1 and 0.05 ml for the remaining 6 doses), was applied to the test site on each rabbit and covered with gauze. The trunk of each animal was then wrapped with impervious plastic sheeting[e] which occluded the test article during the 23 hour contact period.

[a] Hazleton Dutchland, Inc., Denver, PA
[b] Animals were housed in accordance with recommendations contained in DHEW Publication No. 85-23 (NIH): Revised 1985 "Guide For the Care and Use of Laboratory Animals."
[c] Purina Lab Rabbit Chow and rabbits may be offered Alfalfa Cubes for additional roughage.
[d] Draize: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965).
[e] 10 x 12 x .002 Extra Clear polyethylene sleeves, PPC Industries, Inc., Wheeling, IL

At the end of the exposure period the plastic wrappings and all residual test article were removed by washing with water. One hour after removal of the test article, the intact test site was examined and scored for erythema and edema on a graded scale of 0 - 4.

The irritation produced was evaluated by means of the daily average scores for erythema and edema of the intact test site one hour post removal of the test article. The scoring criteria for erythema and edema are shown below.

Scoring Criteria for Skin Reactions

Reaction	Description	Score
Erythema	Barely perceptible (Edges of area not defined)	1
	Pale red in color and area definable	2
	Definite red in color and area well defined.	3
	Beet or crimson red in color	4
Edema	Barely perceptible (Edges of area not defined)	1
	Area definable but not raised more than 1 mm.	2
	Area well defined and raised approximately 1 mm.	3
	Area raised more than 1 mm.	4
	Maximum Primary Irritation Score =	8

The results are presented in Table 1. The protocol, principal personnel involved in the study, composition characteristics and Quality Assurance statement are contained in Appendices I - IV.

Table 1

Repeat Skin Irritation Test - Albino Rabbits

with Hydroxypropylmethylcellulose Gel With

30% Pyridostigmine Bromide + 0.21% Docusate Sodium

1 Hour
Irritation Scores for Intact
Skin Sites after Removal:

Animal Number	Day 1		Day 2		Day 3		Day 4		Day 5	
	ER.	ED.	ER.	ED.	ER.	ED.	ER.	ED.	ER.	ED.
7B302	1	0	1	0	1	0	1	0	2	0
7B305	1	1	2	1	2	1	2	1	2	1
7B297	1	0	1	1	2	1	2	1	2	2
7B271	0	0	1	0	2	1	2	2	2	1
7B292	0	0	0	0	1	1	1	1	1	0
7B270	2	0	2	1	2	2	2	2	3	2
Mean	0.8	0.2	1.2	0.5	1.7	1.0	1.7	1.2	2.0	1.0

Animal Number	Day 6		Day 7	
	ER.	ED.	ER.	ED.
7B302	2	1	2	1
7B305	2	1	X	-
7B297	2	2	2	2
7B271	2	1	2	1
7B292	1	1	1	1
7B270	3	2	2	2
Mean	2.0	1.3	1.8	1.4

Key: ER. = Erythema
ED. = Edema
E = Epithelial Stripping
X = Rabbit 7B305 was found dead at 1 hour post dose administration of dose seven with salivation and tremors noted in this rabbit just prior to death.

APPENDIX I
PROTOCOL

5.

GLP STUDY

TEST: Skin Irritation Test (Repeat Application)

SPONSOR: 3M RIKER Division

CONDUCTED BY: **Safety Evaluation Laboratory, Riker Laboratories, Inc., St. Paul, Minnesota**

TEST ARTICLE: Hydroxypropylmethylcellulose gel containing 30% Pyridostigmine Bromide and 0.2% Docusate Sodium, Lot FN 4589

PROPOSED STARTING/COMPLETION DATE OF TEST: 3/87 - 7/87

TEST SYSTEM: Female New Zealand White Albino Rabbits

SOURCE: Hazleton-Dutchland, Denver, PA

OBJECTIVE: To assess the irritation potential of the test article to the skin of female animals after repeat contact. Rabbits were selected as the test system due to their historical use, sensitivity to irritants, ease of handling and general availability.

METHOD: The animals will be housed in standard wire-mesh cages in temperature and humidity controlled rooms with food[a] and water offered ad libitum. Six animals will be used for this test. Each animal will be assigned a numbered ear tag, which will correspond to a card affixed to the outside of the cage. The test article will be applied to the skin at the same test site on four to seven consecutive days. Prior to the application of the test article, the hair will be clipped from the back and flanks of each animal and one test site selected lateral to the midline of the back. The test site will remain intact. The test article 0.1 ml will be applied to the intact site on each animal, covered with gauze and secured with gauze. The trunk of each animal will then be wrapped with impervious plastic sheeting which will occlude the test article during the 23 hour exposure period. Approximately one hour after removal of the test article, the intact test site will be examined and scored for erythema and edema on a graded scale of 0 to 4[b]. The irritation produced will be evaluated by meaning the scores for erythema and edema of the intact test site one hour post removal of the test article for each application. These values will be assessed for potential cumulative irritation. The raw data generated by the study director and the final report will be stored in the Riker Laboratories' Archive, St. Paul, Minnesota.

[a] Purina Rabbit Chow, Ralston Purina Co., St. Louis, Missouri.
[b] Draize: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965) Published by the Editorial Committee of the Association of Food and Drug Officials of the United States.

Sponsor 3/11/87 Date

Study Director Gene L. Harris 3/16/87 Date

Riker Experiment No. 0387EB0074

Appendix I (concluded)
Deviations and/or Amendments to Protocol

1. A dose of 0.05 ml per animal will be administered starting with dose #2. Reason for change: to avoid mortality of the animals as a result of the test material dose volume.

Gene Harris 3/18/87
Study Director Date

2.

Study Director Date

3.

Study Director Date

4.

Study Director Date

APPENDIX II

Principal Participating Personnel Involved in the Study

Name	Function
G. L. Harris, B.S.	Advanced Toxicologist Study Director
G. E. Hart	Master Laboratory Technician Acute Toxicology
L. O. Wiseth	Sr. Laboratory Technician Acute Toxicology
J. A. Eads	Jr. Laboratory Technician Acute Toxicology
J. L. Allen, Ph.D.	Research Toxicologist Acute Toxicology
G. C. Pecore	Supervisor Animal Laboratory

APPENDIX III

Test and/or Control Article Characterization

for

Hydroxypropylmethylcellulose gel containing 30% pyridostigmine Bromide and 0.2% Docusate Sodium, (FN 4589)

1. The identity strength, uniformity, composition, purity or other pertinent characterizations of the test and/or substances have been determined and documented as of RFA 14201 – 2/27/87.

2. The method of synthesis or origin of the test and control substances, including their amount and the method of bioassay (if applicable) is documented.

   ☐ Yes ☐ No Anita Nith 2/27/87

3. The stability of the test and/or control substances* ~~have been determined~~ ~~or~~ will be determined ~~as of~~ AT the end of the study.

   Anita Nith 2/27/87

The above information and documentation are located in the sponsor's records.

~~Sponsor or~~ Sponsor Representative	Date
Anita Nith	2/27/87

* = Form change

APPENDIX IV

QUALITY ASSURANCE STATEMENT

Acute Toxicology Laboratory Studies

Study No.: 0387EB0074

This short term study was audited by Compliance Audit and the final report examined against the raw data on April 21, 1987. The results of the audit were reported to the study director and to management on April 21, 1987.

In addition to the data audit, different significant phases for studies underway in the Acute Toxicology Laboratory are inspected on a recurring cycle, and the facilities are examined by Compliance Audit on a three month schedule.

D. M. Marboe, Jr.
Compliance Audit

4-21-87
Date

Riker Pathology and Toxicology Department Services Request

(Pathology and Toxicology Department Use Only)

Experiment Number 03..EB0075 807

To: Gene Harris	From: Maria Wutfall	Date 2/11/87
3M Division Riker	Address 270-4S-02	Phone 6-1367 / Riker Project No. 9031000003

Test Article Information

Sample Name and/or I.D. No. (S-26741) - Hydroxypropylmethylcellulose gel containing ... and 0.198% ...	Lot Number FN4570

Test Article Storage Conditions: ☒ Room Temp. ☐ Refrigerate ☐ Other ______

Proposed End Use of Product: Transdermal Patch

The following service is requested on the test article listed above:

Irritation Studies

- ☐ Primary Skin Irritation (Rabbit)
- ☒ Four Day Repeat Skin Irritation (Rabbit)
- ☐ Primary Eye Irritation (Rabbit)
- ☐ Mucous Membrane Irritation (Hamster)
- ☐ Intracutaneous Irritation (Rabbit)
  (Circle extracting mediums required)
  ☐ Saline ☐ 1:20 Ethanol : Saline
  ☐ Cottonseed oil ☐ Peg 400

Sensitization Studies

- ☐ Magnusson - Kligman Maximization (Guinea Pig)
- ☐ Buehler Sensitization (Guinea Pig)

Acute Toxicity Studies

- ☐ Acute Oral Limit Test (1 level in rats)
- ☐ Acute Oral LD_{50} (Rat)
- ☐ Acute Dermal Limit Test (1 level in rabbits)
- ☐ Acute Dermal LD_{50} (Rabbits)
- ☐ Acute I.V. LD_{50} (List species ______)
- ☐ Acute I.P. LD_{50} (List species ______)
- ☐ Acute Systemic Toxicity (USP-Mice)
  (Circle extracting mediums required)
  ☐ Saline ☐ 1:20 Ethanol : Saline
  ☐ Cottonseed oil ☐ Peg 400

Invitro Studies

- ☐ Agar Overlay
- ☐ Cell Growth Inhibition
- ☐ Direct Cell Contact

Special Services Requested

Regulatory Compliance

☒ All studies are to be conducted following the Good Laboratories Practices Act in accordance with ☒ FDA ☐ EPA (FIFRA) ☐ EPA (TSCA) ☐ OECD Governmental Requirements.

☐ All studies are for research and development and are not intended to support a governmental submission or marketing permit.

☐ Other: Explain ______

Authorized By ► Maria Wutfall Date 3/11/87

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Comments

Form 21773 - E - PWO White - Raw Data File Canary - Requestor 3M "Action" 200 Paper

RIKER Experiment No.: 0387EB0075

PROTOCOL

TEST: Skin Irritation Test (Repeat Application) 808

SPONSOR: 3M RIKER Division

CONDUCTED BY: Safety Evaluation Laboratory, Riker Laboratories, Inc., St. Paul, Minnesota

TEST ARTICLE:

PROPOSED STARTING/COMPLETION DATE OF TEST: 3/87 - 7/87

TEST SYSTEM: Female New Zealand White Albino Rabbits

SOURCE: Hazleton-Dutchland, Denver, PA

OBJECTIVE: To assess the irritation potential of the test article to the skin of female animals after repeat contact. Rabbits were selected as the test system due to their historical use, sensitivity to irritants, ease of handling and general availability.

METHOD: The animals will be housed in standard wire-mesh cages in temperature and humidity controlled rooms with food[a] and water offered ad libitum. Six animals will be used for this test. Each animal will be assigned a numbered ear tag, which will correspond to a card affixed to the outside of the cage. The test article will be applied to the skin at the same test site on four to seven consecutive days. Prior to the application of the test article, the hair will be clipped from the back and flanks of each animal and one test site selected lateral to the midline of the back. The test site will remain intact. The test article 0.1 ml will be applied to the intact site on each animal, covered with gauze and secured with gauze. The trunk of each animal will then be wrapped with impervious plastic sheeting which will occlude the test article during the 23 hour exposure period. Approximately one hour after removal of the test article, the intact test site will be examined and scored for erythema and edema on a graded scale of 0 to 4[b]. The irritation produced will be evaluated by meaning the scores for erythema and edema of the intact test site one hour post removal of the test article for each application. These values will be assessed for potential cumulative irritation. The raw data generated by the study director and the final report will be stored in the Riker Laboratories' Archive, St. Paul, Minnesota.

[a] Purina Rabbit Chow, Ralston Purina Co., St. Louis, Missouri

[b] Draize: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965) Published by the Editorial Committee of the Association of Food and Drug Officials of the United States.

Sponsor 3/11/87 Date

Study Director Date

Sensitization Study

with S-26741, Lot 653035

in Albino Guinea Pigs

Experiment No.: 0385MG0411

Conducted At: Pathology and Toxicology
Riker Laboratories, Inc.
St. Paul, Minnesota

Dates Conducted: October 2, 1985 to November 27, 1985

Conducted By:

Gene L. Harris 1/13/86
G. L. Harris, BS Date
Advanced Toxicologist
Study Director

Karen D. O'Malley 1/13/86
K. D. O'Malley, BS Date
Senior Toxicologist
Acute Toxicology

Reviewed By:

K. L. Ebbens 1/14/86
K. L. Ebbens, BS Date
Supervisor, Toxicology Testing

dc: R. T. Catherall
M. W. Downing
K. L. Ebbens
A. K. Mitra
M. J. Westfall (2)
Path/Tox Files

Summary

A sensitization study was conducted from October 2, 1985 to November 27, 1985 at Riker Laboratories, Inc., St. Paul, Minnesota with S-26741, Lot 653035 (Pyridostigmine Bromide). The albino guinea pigs were induced dermally with test article and then subsequently challenged topically. A positive control group (5 animals) using 2,4-Dinitrochlorobenzene, was induced in the same manner as the test article. Subsequent challenge of the test group resulted in (0/9) positive responses while the positive control group showed (5/5) postive responses. The 0% sensitization rate classifies S-26741 as a Grade I or weak sensitizer according to the Magnusson and Kligman rating system. This indicates an extremely low allergenic potential, however, it does not mean that the test article will never be a sensitizer, but rather the probability of sensitization is very low.

Introduction

The object of this study was to determine the sensitization potential of S-26741, Lot 653035 (Pyridostigmine Bromide), in female albino guinea pigs. This study was conducted in accordance with the Food and Drug Administration's Good Laboratory Practice Regulation of 1978. The raw data generated by the Study Director and the final report are stored in the conducting laboratory's archives.

Method and Results

Twenty-two albino guinea pigs of the Hartley strain[a] were used to evaluate the sensitization potential of the test article. The test method was modeled after that of Magnusson, B. and Kligman, an A.M.[b].

An initial rangefinder was undertaken with seven animals to determine an appropriate irritating and sub-lethal concentration for testing. The concentrations used for the testing are shown in Table 1. The induction phase was accomplished in two stages once this irritation had been determined. The initial stage involved six intradermal injections (three per side) using ten animals for the test article groups and five animals for the positive control (DNCB)[c] group. The injections were made in the dorsal shoulder girdle (2 x 4 cm area) which had been clipped free of hair prior to injection. The injection schedule for each side of the animals[d] was as follows: 1) 0.1 ml of Freund's adjuvant (1:1 commercial adjuvant with water), 2) 0.1 ml of the test article at the predetermined concentration by weight in an appropriate vehicle (see Table 1), and 3) 0.1 ml of the test article at the predetermined concentration by weight in the adjuvant. Seven days post intradermal injection, the predetermined topical concentration was applied to a Readi-Bandage®[e] adhesive dressing to saturation and placed on the injection site area, which had been shaved prior to application, and covered with gauze. This in turn was firmly secured with elastic bandage material[f]. The patches were left in place for two days after which the patches and all residual test article were removed.

Thirteen days after the topical application, the hair was clipped from an area on the flank (posterior to the injection site). A sub-irritating concentration of the test article was applied to a Readi-Bandage adhesive dressing in the same fashion as for the topical induction phase and left in place for one day. The challenge sites were evaluated[g] one and two days after removal of the patches on a scale of 0 or 4 for erythema and edema (Table 2).

a Charles River Breeding Laboratories, Inc., Wilmington, MA
b Magnusson, B. & Kligman, A.M. The Identification of Contact Allergens by Animal Assay. The Guinea Pig Maximization Test. J. Invest. Derm., 52-268 (1969)
c 2.4-Dinitrochlorobenzene, Sigma Chemical Co., St. Louis, MO
d Difco Labs, Inc., Detroit, MI
e Readi-Bandage®, Parke, Davis & Co., Detroit, MI
f ElastoPlast®, Biersdorf, Inc., S. Norwalk, CT Lawn NJ
g Draize: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics, (1965)

The grading system used to arrive at a descriptive rating is located below.

Sensitization Rate (%)	Grade	Classification
0 - 8	I	Weak
9 - 28	II	Mild
29 - 64	III	Moderate
65 - 80	IV	Strong
81 - 100	V	Extreme

The results of the study are shown in Tables 3 - 5. The protocol, principal personnel involved in the study, composition characteristics and Quality Assurance statement are contained in Appendices I-IV.

TABLE 1

Maximization Sensitization Study - Albino Guinea Pigs

Treatment Procedure

Test Article	Number of Animals Evaluated	Induction Phase Concentration of Injection	Induction Phase Concentration of Topical	Challenge Phase Concentration of Topical
S-26741	10	0.125%	25%	25%
DNCB	5	0.1	0.1%	0.5%

TABLE 2

MAXIMIZATION SENSITIZATION TEST - ALBINO GUINEA PIGS

Scoring Criteria for Skin Reactions

Reaction	Description	Score
Erythema	Barely perceptible (Edges of area not defined)	1
	Pale red in color and area definable	2
	Definite red in color and area well defined.	3
	Beet or crimson red in color	4
Edema	Barely perceptible (Edges of area not defined)	1
	Area definable but not raised more 1 mm.	2
	Area well defined and raised approximately 1 mm.	3
	Area raised more than 1 mm.	4
	Maximum Primary Irritation Score	= 8

TABLE 3

Maximization-Sensitization Test - Albino Guinea Pigs

Rangefinder with S-26741

RESULTS

Animal Number	Concentration Tested[a]	Route	Irritation Score for Skin Sites 24 Hours After Sample Removal Erythema	Edema
5G4390	Undiluted	Topical	X	X
5G4389	50%	Topical	X	X
5G4392	25%	Topical	X	X
5G4391	10%	Topical	X	X
5G4388[b]	5%	Topical	0	0
5G4390	5%	Injection	X	X
5G4389	4%	Injection	X	X
5G4392	3%	Injection	X	X
5G4391	2%	Injection	X	X
5G4388[b]	1%	Injection	0	0
5G5145[c]	0.5%	Injection	0	0
5G5146[c]	0.25%	Injection	0	0

[a] Sterile water, Travenol, Lot 4G720F4 was used as the vehicle

X = Animal died within four hours of dosing and had convulsions just prior to death.

[b] This animal was prostrate for about four hours after dosing.

[c] This animal had no compound related pharmacotoxic signs.

TABLE 4

Maximization Sensitization Test - Albino Guinea Pigs

with S-26741[a]

RESULTS

Animal Number	Irritation Scores for Skin Sites After Sample Removal			
	One Day		Two Days	
	Erythema	Edema	Erythema	Edema
5G5230	0	0	0	0
5G5231	X	X	X	X
5G5232	0	0	0	0
5G5152	0	0	0	0
5G5158	0	0	0	0
5G5236	0	0	0	0
5G5237	0	0	0	0
5G5238	0	0	0	0
5G5164	0	0	0	0
5G5167	0	0	0	0

Percent Sensitized: 0

X = This animal was found dead two days post initial induction and this death is presumed to be compound related.

[a] Sterile water, Travenol, Lot 4G720F4 was used as the vehicle.

TABLE 5

Maximization Sensitization Test - Albino Guinea Pigs

with DNCB[a]

RESULTS

Animal Number	Irritation Scores for Skin Sites After Sample Removal			
	One Day		Two Days	
	Erythema	Edema	Erythema	Edema
5G5242	2	1	2	1
5G5243	1	0	1	1
5G5166	2	1	2	1
5G5165	2	1	2	1
5G5126	2	1	2	1

Percent Sensitized: 100

[a] Eastman Kodak, Sigma Chemical, Lot 44F-0565 dissolved in Propylene Glycol, Kodak, Lot A12B.

APPENDIX I
PROTOCOL

Riker Experiment No.: 0385MG0411

818

TEST: Magnusson-Kligman Maximization-Sensitization Test

SPONSOR: 3M Riker Division

CONDUCTED BY: **Safety Evaluation Laboratory, Riker Laboratories, Inc., St. Paul, Minnesota** RF. E.M.

TEST ARTICLE: S-26741, Pyridostigmine Bromide, LOT 6583035

CONTROL ARTICLE: Footnote c

PROPOSED STARTING/COMPLETION DATE OF TEST: 10/85 - 1/86

TEST SYSTEM: Hartley Strain, Guinea Pig

SOURCE: Charles River Breeding Laboratories, Wilmington, Massachusetts

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OBJECTIVE: To determine the sensitization potential of the test article. Guinea pigs will be used as the test system due to their historical use and ease of handling.

METHOD: The method will be similar to that of Magnusson and Kligman[a]. The animals will be housed in standard cages in temperature and humidity controlled rooms with food[b] and water *ad libitum*. The animals' numbers will be placed on cards affixed to the outside of their cages. An initial rangefinder will be conducted using 5 animals to determine the appropriate concentrations of the test article to be used in the test. The test will be conducted in two stages; an induction phase and a challenge phase. The induction phase will consist of six intradermal injections (3 per side) in each of 10 animals, for the test article, and in each of 5 animals for the positive control[c]. The injections will be made in the dorsal shoulder girdle (2 x 4 cm area) which will be clipped free of hair prior to injection. The injection schedule will be (1) 0.1 ml of Freund's Adjuvant[d] (1:1 commercial adjuvant with water), (2) 0.1 ml of the test article at the appropriate concentration by weight with the adjuvant, and (3) 0.1 ml of the test article at the appropriate concentration (in Sterile Water). Seven days post intradermal injection a Readi-Bandage®[e], saturated (approximately 0.1 ml) with an appropriate concentration of the test article, will be placed near the injection site area, which will be clipped free of hair prior to application, and secured with gauze wrap (if the test article is non-irritating, the area will be pre-treated with 10% SLS[f] in petrolatum 1 day prior to the patches being applied). This in turn will be firmly secured with elastic bandage. The test article will be left in place for 2 days, after which the wrappings and all residual test article will be removed. Two weeks after the topical application, the hair will be removed from an area on the flank (posterior to the induction sites). A subirritating concentration (approximately 0.1 ml) of the test article will be applied on a Readi-Bandage®[e], covered with an opened 2" x 2" gauze patch, secured with elastic bandage and left in place for 1 day. The challenge site will be evaluated 1 and 2 days after removal of the wrapping and all residual test article. The skin reactions will be scored on the basis of 0 to 4[g] and a descriptive rating assigned[a]. All raw data generated by the study director and the final report will be stored in the Riker Laboratories' Archive, St. Paul, Minnesota.

[a] Magnusson, B. & Kligman, A.M.; The Identification of Contact Allergens by Animal Assay. The Guinea Pig Maximization Test. J. Invest. Derm. 53:268 (1969)
[b] Purina Guinea Pig Chow, Ralston Purina Co., St. Louis, Missouri
[c] 2,4-Dinitrochlorobenzene, Sigma Chemical Co., St. Louis, Missouri
[d] Difco Laboratories, Inc., Detroit, Michigan
[e] Parke, Davis & Co., Detroit, Michigan
[f] Sodium Lauryl Sulfate, Sigma Chemical Co., St. Louis, Missouri
[g] Draize: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965) Published by the Editorial Committee of the Association of Food and Drug Officials of the United States.

K L Ekberg 9/16/85
Sponsor Date

Glen L. Harris 9/18/85
Study Director Date

Riker Experiment No. 0385MG0411

Appendix I (concluded)
Deviations and/or Amendments to Protocol

819

1. Because of the mortalities observed in the rangefinding study, two additional guinea pigs will be added to the rangefinding study and given an injection dose only. This is being done to see if smaller doses will cause any systemic effects.

G. L. Harris — 10/30/85
Study Director — Date

2. The challenge dose will be administered 13 days after topical induction. Reason for change: To accommodate scheduling problems over the Thanksgiving Holiday.

G. L. Harris — 11/24/85
Study Director — Date

3. _____

Study Director — Date

4. _____

Study Director — Date

5. _____

Study Director — Date

APPENDIX II

Principal Participating Personnel Involved in the Study

Name	Function
G. L. Harris, BS	Advanced Toxicologist Study Director
G. E. Hart	Sr. Laboratory Technician Acute Toxicology
K. D. O'Malley, BS	Senior Toxicologist Acute Toxicology
K. L. Ebbens, BS	Supervisor Toxicology Testing
G. C. Pecore	Supervisor Animal Laboratory

APPENDIX III - A

Test and/or Control Article Characterization

for

S-26741, (PYRIDOSTIGMINE BROMIDE), Lot# 653035

1. The identity strength, uniformity, composition, purity or other pertinent characterizations of the test and/or control substances have been determined and documented as of 8/19/85.

2. The method of synthesis or origin of the test and control substances, including their amount and the method of bioassay (if applicable) is documented.

yes X no ___ ~~(NOT APPLICABLE)~~ RE 6H 1/30/86

3. The stability of the test and/or control substances have been determined or will be determined as of ___.

Raw material stability (NOT REQUIRED FOR ACUTE STUDIES) - SEE PATHOLOGY/TOX SOP's

The above information and documentation are located in the sponsor's records.

Sponsor — Date 1/30/86

Form 19793 PWO

822

3M

Riker St. Paul Drug Clearance Certificate

☒ Original Clearance ☐ Re-Clearance

Purpose: Reference Standard		
Sample Description: Pyridostigmine Bromide		
Compound Lot No: Hoffman-LaRoche Lot #653035	Batch Size: 200 gm	RFA: 11055
Reference Standard Lot: -	Previous References: -	

Test Results ☒ Full Clearance ☐ Selected Tests

Assay: 99.19% (on the dried basis)

Loss on Drying: 0.51%

Identification:

Infrared Spectrum: Spectrum IR 1492 agrees with USP Reference Standard Spectrum IR 1491.

Ultraviolet Spectrum: Spectrum UV 1565 agrees with USP Reference Standard Spectrum UV 1564.
Respective absorptivity 103.0% of USP Reference Standard.

Identification C: Responds to identification test.

Identification D: Responds to test for Bromide.

Melting Range: 154.2° - 155.0°

Residue on Ignition: 0

Note: Specifications or reference value in parenthesis

* Not formal clearance specification

Comments

Reference USP XXI

Analytical Review	Date	Quality Control Approval	Date
C. A. Kolars Charles A Kolars	10-14-85	Eugene R Bell	9 JAN 86

Form 22167 Rev1

APPENDIX IV

QUALITY ASSURANCE STATEMENT

Acute Toxicology Laboratory Studies

Study No.: 0385MG0411

This short term study was audited by Compliance Audit and the final report examined against the raw data on February 3, 1986. The results of the audit were reported to the study director and to management on February 3, 1986.

In addition to the data audit, different significant phases for studies underway in the Acute Toxicology Laboratory are inspected weekly on a recurring cyle, and the facilities are examined by Compliance Audit on a three month schedule.

Gale E. VonBuskirk
Compliance Audit

February 3, 1986
Date

Pathology and Toxicology
Riker Laboratories, Inc.

Building 270-3S-05, 3M Center
St. Paul, Minnesota 55144-1000

COMPANY CONFIDENTIAL

3M

Sensitizaton Study

with 50% S-26741 + 0.33% Sodium Lauryl Sulfate in a Microporous Membrane

in Yorkshire Swine

Riker Experiment No: 0386MS0737

Conducted At: Pathology and Toxicology
Riker Laboratories, Inc.

Dates Conducted: December 10, 1986 to January 7, 1987

Conducted By: Gene L. Harris 3/3/87
G. L. Harris, B.S. Date
Advanced Toxicologist
Study Director and Acute Toxicity
Study Coordinator

Reviewed By: J.L. Allen 3-4-87
J. L. Allen, Ph.D. Date
Diplomate, A.B.T.
Research Toxicologist

dc: J. L. Allen
R. T. Catherall
C. F. Chesney
M. W. Downing
N. M. Marecki
M. J. Westfall (2)
Tech. Doc. Cntr.
Path/Tox File (S-26741)

Summary

A sensitization study was conducted from December 10, 1986 to January 7, 1987 at Riker Laboratories, Inc., St. Paul, Minnesota with 50% S-26741 + 0.33% sodium lauryl sulfate in a microporous membrane. Two Yorkshire swine received six topical induction applications of the potential antigen and were then subsequently challenged topically 14 days post induction. The average score following the challenge dose was 3.5 out of a maximum score of 4.0 compared to an average score of 0.0 after the first induction dose. A 75% increase in irritation was noted when the challenge dose score was compared to the average score for all six induction applications (2.0/4.0 see Table 2). Both swine were also challenged with 50% S-26741 gel (without sodium lauryl sulfate) which resulted in an average score of 2.9/4.0 and with 0.33% sodium lauryl sulfate gel (without S-26741) which resulted in an average score of 0/4.0. Based on these results it is possible that the test material (S-26741) may be a potential skin sensitizing agent. However, the results are equivocal because this study did not include a positive control group and the animal species (Yorkshire swine) chosen for this study is poorly understood as a model for skin sensitization studies. It is possible that the use of Freund's adjuvant may have contributed to the increase in severity of irritation following the challenge dose. In addition, the severe dermal irritation noted in the swine at the last induction application could have lowered the threshold for irritation reactions to occur at a challenge site in the animals (Marzulli & Maibach, 1983). Therefore, the challenge dose score may be a false positive with respect to skin sensitization.

The results of this study indicate that the test material should be investigated further as a potential skin sensitizing agent.

Introduction

The object of this study was to explore the sensitization potential of 50% S-26741 + 0.33% sodium lauryl sulfate in a microporous membrane in Yorkshire swine. This study was conducted for research and development purposes and is, therefore, not regulated by the Food and Drug Administration's Good Laboratory Practice Regulation of 1978, although the standard operating procedures of this laboratory adhere to the general principles of this regulation. The raw data generated by the Study Director and the final report are stored in the conducting laboratory's archives.

Method and Results

Two Yorkshire X swine[a] were used to evaluate the sensitization potential of the test article. All animals were held under quarantine for several days prior to testing and only animals which appeared to be in good health and suitable as test animals at the initiation of the study were used. The swine were housed in temperature and humidity controlled rooms and permitted a standard laboratory diet[b] and water ad libitum. The test method was a modification of Buehler[c]. The induction phase consisted of six topical applications of the test article[d] on the dorsal shoulder girdle of two animals, which had been clipped free of hair prior to the application. The initial topical application also included two 0.2 ml intradermal injections of Freund's Adjuvant[e] (1:1 commercial adjuvant with water) in an area close to where the test article was immediately applied. The patches were secured and this in turn was overwrapped with elastic-like bandage material[f]. The patches were left in place for approximately a 24 hour contact period after which the patches and all residual test article were removed. Six topical applications of the test article at three applications per week (Monday, Wednesday and Friday) were applied to the dorsal shoulder girdle of the two test swine. Each of the six induction applications were scored on a basis of 0 to 4[g] at 1 hour and 24 hours after test article removal. Fourteen days after the final induction application, the hair was removed from an area on the flank (posterior to the induction site) and the test article was applied in the same fashion as it was applied during the induction phase. This was left in place for one day. Two additional materials were also administered to the flanks of the animals, only at the challenge dose application. These materials were 50% S-26741 gel (without sodium lauryl sulfate) and 0.33% sodium lauryl sulfate gel (without S-26741). All challenge sites were evaluated one day and two days after removal of the patches, on a scale of 0 to 4, for erythema and edema[g] (Table 1).

The protocol and principal personnel involved in the study are contained in Appendices I - IV.

[a] Ben Bartusek, Jr., New Prague, Minnesota
[b] Pig Starter 4-4-4, Ralston Purina, St. Louis, MO
[c] Buehler, EV: Delayed Contact Hypersensitivity in the Guinea Pig. Arch. Dermat. 91:171 (1965).
[d] A 40 square centimeter microporous membrane patch containing 50% S-26741 + 0.33% sodium lauryl sulfate in a gel-like material.
[e] Difco Labs, Inc., Detroit, MI
[f] SCOTCHRAP®, 3M, St. Paul,
[g] Draize: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics, (1965)

TABLE 1

SENSITIZATION TEST - YORKSHIRE SWINE

Scoring Criteria for Skin Reactions

Reaction	Description	Score
Erythema	Barely perceptible (Edges of area not defined)	1
	Pale red in color and area definable	2
	Definite red in color and area well defined.	3
	Beet or crimson red in color	4
Edema	Barely perceptible (Edges of area not defined)	1
	Area definable but not raised more 1 mm.	2
	Area well defined and raised approximately 1 mm.	3
	Area raised more than 1 mm.	4
	Maximum Primary Irritation Score	= 8

TABLE 2-A

Individual Average Calculations
Test Article: 40 Square cm Microporous Membrane Patch
50% S-26741 + 0.33% Sodium Lauryl Sulfate In A Gel-Like Material

Animal #	Dose #	1		2		3		4		5		6		6 Dose Average	Challenge Dose	
	Hour After Removal	1	24	1	24	1	24	1	24	1	24	1	24		1	24
6S-37	Erythema	0	0	1	1	2	3	4	4	4	4	4	4		3	4
	Edema	0	0	1	0	2	2	2	3	4	4	4	4		3	4
	Average	0.0		0.8		2.3		3.3		4.0		4.0		2.4	3.5	
6S-38	Erythema	0	0	0	0	1	0	2	3	4	4	4	4		3	4
	Edema	0	0	0	0	0	0	1	2	3	3	4	4		3	4
	Average	0.0		0.0		0.3		2.0		3.5		4.0		1.6	3.5	

0/4.0	2.0/4.0	3.5/4.0
Group Initial Dose Average	Group 6 Dose Average	Group Challenge Dose Average

TABLE 2-B

Test Article: 50% S-26741 In A Gel-Like Material (Challenge Dose Only)

Animal #		Challenge Dose 1	24
6S-37	Erythema	3	3
	Edema	3	3
	Average	3.0	

Animal #		Challenge Dose 1	24
6S-38	Erythema	3	3
	Edema	3	2
	Average	2.8	

2.9/4.0

Group Challenge Dose Average

Test Article: 0.33% Sodium Laryl Sulfate In A Gel-Like Material (Challenge Dose Only)

Animal #		Challenge Dose 1	24
6S-37	Erythema	0	0
	Edema	0	0
	Average	0.0	

Animal #		Challenge Dose 1	24
6S-38	Erythema	0	0
	Edema	0	0
	Average	0.0	

0.0/4.0

Group Challenge Dose Average

REFERENCES

Marzulli F. and Maibach H. 1983. Dermatotoxicology, p. 273, Hemisphere Publishing Corp.

TEST: Modified Buehler[a] Sensitization Test

SPONSOR: 3M RIKER Division

CONDUCTED BY: Pathology and Toxicology Department, Riker Laboratories, Inc., St. Paul, Minnesota

TEST ARTICLE: 50% S-26741 + 0.33% Sodium Lauryl Sulfate in a Microporous Membrane

CONTROL ARTICLE: (Due to animal availability no positive control will be used).

PROPOSED STARTING/COMPLETION DATE OF TEST: 12/86 - 3/87

TEST SYSTEM: Yorkshire X Swine

AGE: 3 - 5 Months

SOURCE: Ben Bartusek, Jr., New Prague, Minnesota

OBJECTIVE: To determine the sensitization potential of the test article. Yorkshire X pigs will be used as the test system, because a possible sensitization response has been observed in Yorkshire X pigs on a prior study.

METHOD: The method will be a modification of Buehler[a]. The animals will be housed individually in runs in temperature and humidity controlled rooms with food[b] and water offered *ad libitum*. The animals' numbers will be placed on cards affixed to the outside of their cages and on tags affixed to the pigs ears. The test will be conducted in two phases; an induction phase and a challenge phase. The induction phase will consist of nine topical applications of the test article[c] at 3 applications per week (Monday, Wednesday and Friday) in the hind dorsal back area, which will be clipped free of hair prior to the application procedure. The initial application will consist of two injections of 0.2 ml (per injection - one/side) of Freund's Adjuvant[d] (1:1 commercial adjuvant with water) in each of the 2 animals that will be administered the test article. The test article will be placed near the injection sites and firmly secured. The test article will be left in place for approximately 24 hours, after which all residual test material will be removed. The subsequent applications will be done in the same manner as the initial application excluding the injection of adjuvant. Each of the 9 induction applications will be scored on a basis of 0 to 4[e] at 1 hour and 24 hours after test article removal. Two weeks after the induction phase is complete, the hair will be removed from both flanks (avoiding the induction site). The challenge phase will consist of one application of three separate test articles. In addition to the original test article used for induction, a microporous membrane containing 50% S-26741 only will be applied and a microporous membrane containing 0.33% Sodium Lauryl Sulfate only will be applied. All challenge articles will be applied

continued

and secured and left in place for 24 hours. The challenge sites will be evaluated 1 and 2 days after removal of the test articles. The skin reactions will be scored on the basis of 0 to 4[e]. At the discretion of the study director punch biopsies of the test sites may be taken during the study and/or additional test articles may be administered during the challenge phase of this study. All raw data generated by the study director and the final report will be stored in the Riker Laboratories' Archive, St. Paul, Minnesota.

[a] Buehler, EV;Delayed Contact Hypersensitivity in the Yorkshire X Pig, Arch. Dermat. 91:171 (1965)
[b] Purina Pig Chow, Ralston Purina Co., St. Louis, Missouri
[c] The test article dose will be ~2 grams S-26741 per patch.
[d] Difco Laboratories, Inc., Detroit Michigan
[e] Draize: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965)

Sponsor Date 12/8/86

Study Director Date 12/8/86

Riker Experiment No. 0386MS0737

Appendix I (concluded)
Deviations and/or Amendments to Protocol

1. Due to the increasingly severe skin irritation and clinical signs, the induction phase will be limited to the first six applications.

J.L. Allen for Gene Harris 12/22/86
Study Director Date

2.

Study Director Date

3.

Study Director Date

4.

Study Director Date

APPENDIX II

Principal Participating Personnel Involved in the Study

Name	Function
G. L. Harris, B.S.	Advanced Toxicologist Study Director
G. E. Hart	Master Laboratory Technician Acute Toxicology
L. O. Wiseth	Sr. Laboratory Technician Acute Toxicology
J. A. Eads	Jr. Laboratory Technician Acute Toxicology
J. L. Allen, Ph.D.	Research Toxicologist Acute Toxicology
G. C. Pecore	Supervisor Animal Laboratory

APPENDIX III

Composition Characteristics

This study is not regulated by the Good Laboratory Practice Act of 1978 and therefore information pertaining to composition characteristics is not applicable for inclusion in this study.

APPENDIX IV

Quality Assurance Statement

This study is not officially regulated by the Good Laboratory Practice Regulation of 1978, and therefore a statement signed and prepared by the Compliance Audit department is not applicable.

The standard operating procedures of this laboratory does adhere to the general principles of this regulation. The Compliance Audit department does inspect differenct significant phases for studies underway in the Acute Toxicology Laboratory on a recurring cycle, and the facilities are examined on a three month schedule. In addition a select number of Research & Development studies are routinely picked at random from the Archives by the Compliance Audit department for review.

Pathology and Toxicology
Riker Laboratories, Inc.

Building 270-3S-05, 3M Center
St. Paul, Minnesota 55144-1000

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Sensitizaton Study

with 50% S-26741 + 0.33% Sodium Lauryl Sulfate

In Solution With Water And 5% Glycerin

in Albino Guinea Pigs

Riker Experiment No: 0386MG0769

Conducted At: Pathology and Toxicology
Riker Laboratories, Inc.

Dates Conducted: December 10, 1986 to January 8, 1987

Conducted By: Gina L. Harris 1/20/87
G. L. Harris, B.S. Date
Advanced Toxicologist
Study Director and Acute Toxicity
Study Coordinator

Reviewed By: J Allen 1-20-87
J. L. Allen, Ph.D. Date
Diplomate, A.B.T.
Research Toxicologist

dc: J. L. Allen
R. T. Catherall
C. F. Chesney
M. W. Downing
N. M Marecki
M. J. Westfall
Tech. Doc. Cntr.
Path/Tox File (S-26741)

Summary

A sensitization study was conducted from December 10, 1986 to January 8, 1987 at Riker Laboratories, Inc., St. Paul, Minnesota with 50% S-26741 + 0.33% sodium lauryl sulfate in solution with water and 5% glycerin. The albino guinea pigs were induced intradermally with test article and then subsequently challenged topically. A positive control group (5 animals) using 2,4-Dinitrochlorobenzene, was induced in the same manner as the test group. Subsequent challenge of the test group resulted in 0/10 positive responses while the positive control group showed 5/5 positive responses. The 0% sensitization rate classifies 50% S-26741 + 0.33% sodium lauryl sulfate in solution with water and 5% glycerin as a Grade I or weak sensitizer according to the Magnusson and Kligman rating system. This indicates an extremely low allergenic potential, however, it does not mean that the test article will never be a sensitizer, but rather the probability of sensitization is very low. It should be noted that the intradermal injections of S-26741 dosed at 0.125% is the maximum concentration of drug that can be delivered intradermally without causing mortalities (see study #0385MG0411).

Introduction

The object of this study was to determine the sensitization potential of 50% S-26741 + 0.33% sodium lauryl sulfate in solution with water and 5% glycerin in female albino guinea pigs. This study was conducted for research and development purposes and is, therefore, not regulated by the Food and Drug Administration's Good Laboratory Practice Regulation of 1978, although the standard operating procedures of this laboratory adhere to the general principles of this regulation. The raw data generated by the Study Director and the final report are stored in the conducting laboratory's archives.

Method and Results

Twenty-two albino guinea pigs of the Hartley strain[a] were used to evaluate the sensitization potential of the test article. The test method was modeled after that of Magnusson, B. and Kligman, an A.M.[b].

An initial rangefinder was undertaken with two animals to determine an appropriate irritating concentration for testing. The concentrations used for the testing are shown in Table 1. The induction phase was accomplished in two stages once this irritation had been determined. The initial stage involved six intradermal injections (three per side) using ten animals for the test article groups and ten animals for the positive control (DNCB)[c] group. The injections were made in the dorsal shoulder girdle (2 x 4 cm area) which had been clipped free of hair prior to injection. The injection schedule for each side of the animals was as follows: 1) 0.1 ml of Freund's adjuvant[d] (1:1 commercial adjuvant with water), 2) 0.1 ml of the test article at the predetermined concentration by weight in an appropriate vehicle (see Table 1), and 3) 0.1 ml of the test article at the predetermined concentration by weight with the adjuvant. Seven days post intradermal injection, the predetermined topical concentration was applied to a Readi-Bandage[e] adhesive dressing to saturation and placed on the injection site area, which had been shaved prior to application, and covered with gauze. This in turn was firmly secured with elastic bandage material[f]. The patches were left in place for two days after which the patches and all residual test article were removed.

Fourteen days after the topical application, the hair was clipped from an area on the flank (posterior to the injection site). A sub-irritating concentration of the test article was applied to a Readi-Bandage adhesive dressing in the same fashion as for the topical induction phase and left in place for one day. The challenge sites were evaluated[g] one and two days after removal of the patches on a scale of 0 or 4 for erythema and edema (Table 2).

[a] Hazleton Dutchland, Inc., Denver PA
[b] Magnusson, B. & Kligman, A.M. The Identification of Contact Allergens by Animal Assay. The Guinea Pig Maximization Test. J. Invest. Derm., 52-268 (1969)
[c] 2.4-Dinitrochlorobenzene, Sigma Chemical Co., St. Louis, MO
[d] Difco Labs, Inc., Detroit, MI
[e] Readi-Bandage , Parke, Davis & Co., Detroit, MI
[f] ElastoPlast , Biersdorf, Inc., S. Norwalk, CT Lawn NJ
[g] Draize: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics, (1965)

The grading system used to arrive at a descriptive rating is located below.

Sensitization Rate (%)	Grade	Classification
0 - 8	I	Weak
9 - 28	II	Mild
29 - 64	III	Moderate
65 - 80	IV	Strong
81 - 100	V	Extreme

The results of the study are shown in Tables 3 - 5. The protocol, principal personnel involved in the study, composition characteristics and Quality Assurance statement are contained in Appendices I-IV.

TABLE 1

Maximization Sensitization Study - Albino Guinea Pigs

Treatment Procedure

Test Article	Number of Animals Evaluated	Induction Phase Concentration of Injection	Induction Phase Concentration of Topical	Challenge Phase Concentration of Topical
50% S-26741 + 0.33% Sodium Lauryl Sulfate in Solution with Water and 5% glycerin	10	0.125%	Undiluted	Undiluted
DNCB	5	0.1%	0.1%	0.05%

TABLE 2

MAXIMIZATION SENSITIZATION TEST - ALBINO GUINEA PIGS

Scoring Criteria for Skin Reactions

Reaction	Description	Score
Erythema	Barely perceptible (Edges of area not defined)	1
	Pale red in color and area definable	2
	Definite red in color and area well defined.	3
	Beet or crimson red in color	4
Edema	Barely perceptible (Edges of area not defined)	1
	Area definable but not raised more 1 mm.	2
	Area well defined and raised approximately 1 mm.	3
	Area raised more than 1 mm.	4
	Maximum Primary Irritation Score	= 8

TABLE 3

Maximization-Sensitization Test - Albino Guinea Pigs

Rangefinder with 50% S-26741 + 0.33% Sodium Lauryl Sulfate In Solution With Water And 5% Glycerin

RESULTS

Animal Number	Concentration Tested[a]	Route	Irritation Score for Skin Sites 24 Hours After Sample Removal Erythema	Edema
6G4297	0.125%	Intradermal Injection	1	1
6G4303	0.125%	Topical	0	0

[a] Sterile water, Vedco, Lot 05557 was used as the vehicle

TABLE 4

Maximization Sensitization Test - Albino Guinea Pigs

with 50% S-26741 + 0.33% Sodium Lauryl Sulfate in

Solution With Water And 5% Glycerin

RESULTS

Animal Number	Irritation Scores for Skin Sites After Sample Removal			
	One Day		Two Days	
	Erythema	Edema	Erythema	Edema
6G4910	0	0	0	0
6G4916	0	0	0	0
6G4922	0	0	0	0
6G4928	0	0	0	0
6G4934	0	0	0	0
6G4911	0	0	0	0
6G4917	0	0	0	0
6G4923	0	0	0	0
6G4929	0	0	0	0
6G4935	0	0	0	0

Percent Sensitized: 0

TABLE 5

Maximization Sensitization Test - Albino Guinea Pigs

with DNCB[a]

RESULTS

Animal Number	Irritation Scores for Skin Sites After Sample Removal			
	One Day		Two Days	
	Erythema	Edema	Erythema	Edema
6G4912	1	0	1	0
6G4918	2	1	1	0
6G4924	2	1	2	1
6G4930	1	0	1	0
6G4936	1	0	1	0

Percent Sensitized: 100

[a] Eastman Kodak, Sigma Chemical, Lot 44F-0565

APPENDIX I

PROTOCOL

Riker Experiment No.: 0386MG0769

846

9.

TEST: Magnusson-Kligman Maximization-Sensitization Test NON-GLP STUDY

SPONSOR: 3M Riker Division

CONDUCTED BY: **Pathology and Toxicology Department, Riker Laboratories, Inc., St. Paul, Minnesota**

TEST ARTICLE: SC[illegible] 1% Sodium Lauryl Sulfate in water and 5% Glycerin

CONTROL ARTICLE: 2, 4 - dinitrochlorobenzene

PROPOSED STARTING/COMPLETION DATE OF TEST: 12/86 – 4/87

TEST SYSTEM: Hartley Strain, Guinea Pig

AGE: 6-~~10~~ 12 weeks of age

SOURCE: Charles River Breeding Laboratories, Wilmington, Massachusetts

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OBJECTIVE: To determine the sensitization potential of the test article. Guinea pigs will be used as the test system due to their historical use and ease of handling.

METHOD: The method will be similar to that of Magnusson and Kligman[a]. The animals will be housed in standard cages in temperature and humidity controlled rooms with food[b] and water *ad libitum*. The animals' numbers will be placed on cards affixed to the outside of their cages. An initial rangefinder may be conducted if necessary to determine the appropriate concentrations of the test article to be used in the test. The test will be conducted in two stages; and induction phase and a challenge phase. The induction phase will consist of six intradermal injections (3 per side) in each of 10 animals, for the test article, and in each of 5 animals for the positive control[c]. The injections will be made in the dorsal shoulder girdle which will be clipped free of hair prior to injection. The injection schedule will be (1) 0.1 ml of Freund's Adjuvant[d] (1:1 commercial adjuvant with water), (2) 0.1 ml of the test article at the appropriate concentration with the adjuvant, and (3) 0.1 ml of the test article at the appropriate concentration (in WATER). One week post intradermal injection an appropriate concentration and volume of the test article, will be placed near the injection site area and firmly secured. The area may be pre-treated with 10% SLS[e] in petrolatum 1 day prior to the patches being applied if the test article produces no irritation. The test article will be left in place for 2 days, after which the wrappings and all residual test article will be removed. Two weeks after the topical application, the hair will be removed from an area on the flank (posterior to the induction sites). A subirritating concentration of the test article will be applied, secured, and left in place for 1 day. The challenge site will be evaluated 1 and 2 days after removal of the wrapping and all residual test article. The skin reactions will be scored on the basis of 0 to 4[f] and a descriptive rating assigned[a]. All raw data generated by the study director and the final report will be stored in the Riker Laboratories' Archive, St. Paul, Minnesota.

a Magnusson, B. & Kligman, A.M.; The Identification of Contact Allergens by Animal Assay. The Guinea Pig Maximization Test. J. Invest. Derm. 53:268 (1969)
b Purina Guinea Pig Chow, Ralston Purina Co., St. Louis, Missouri
c 2,4-Dinitrochlorobenzene, Sigma Chemical Co., St. Louis, Missouri
d Difco Laboratories, Inc., Detroit, Michigan
e Sodium Lauryl Sulfate, Sigma Chemical Co., St. Louis, Missouri
f Draize: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965) Published by the Editorial Committee of the Association of Food and Drug Officials of the United States.

M. Chesney 12-12-86
Sponsor Date

Lynn L. Harris 12/12/86
Study Director Date

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APPENDIX II

Principal Participating Personnel Involved in the Study

Name	Function
G. L. Harris, B.S.	Advanced Toxicologist Study Director
G. E. Hart	Master Laboratory Technician Acute Toxicology
L. O. Wiseth	Sr. Laboratory Technician Acute Toxicology
J. A. Eads	Jr. Laboratory Technician Acute Toxicology
J. L. Allen, Ph.D.	Research Toxicologist Acute Toxicology
G. C. Pecore	Supervisor Animal Laboratory

APPENDIX III

Composition Characteristics

This study is not regulated by the Good Laboratory Practice Act of 1978 and therefore information pertaining to composition characteristics is not applicable for inclusion in this study.

APPENDIX IV

Quality Assurance Statement

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Pathology and Toxicology
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Building 270-3S-05, 3M Center
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850

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3M

Sensitizaton Study

with Hydroxypropylmethylcellulose Gel Containing 50%

Pyridostigmine Bromide

in Albino Guinea Pigs

Riker Experiment No: 0387MG0051

Conducted At: Pathology and Toxicology
Riker Laboratories, Inc.

Dates Conducted: March 4, 1987 to April 2, 1987

Conducted By: Tom L. Harris 4/22/87

G. L. Harris, B.S. Date
Advanced Toxicologist
Study Director and Acute Toxicity
Study Coordinator

Reviewed By: J Allen 4-22-87

J. L. Allen, Ph.D. Date
Diplomate, A.B.T.
Research Toxicologist

dc: R.T. Catherall
M.W. Downing
N.M. Marecki
M.J. Westfall (2)
Tech. Doc. Center
Path/Tox File

Summary

A skin sensitization study was conducted from March 4, 1987 to April 2, 1987 at Riker Laboratories, Inc., St. Paul, Minnesota. The results indicate that hydroxypropylmethylcellulose gel containing 50% pyridostigmine bromide is a potentially moderate sensitizer with positive responses noted in 4/9 animals. An individual animal was considered positive in this test if irritation was noted in the animal at the challenge dose evaluation. The initial induction dose application score for each animal in this study was zero.

All animals in all groups in this study received two, 0.1 ml intradermal injections of Freund's complete adjuvant just prior to the initial induction dose.

Ten female albino guinea pigs in the test article group received six topical induction applications of the potential antigen and subsequently challenged topically 14 days post induction. One animal in the test article group was found dead after dose administration of the challenge dose application. Tremors and salivation were noted in one animal at approximately one hour after dose administration of induction dose # 6. The animal found dead is not included in the final evaluation, although the irritation scores for this animal are listed in Table 2. Due to the adverse effects noted in the test article-treated animals by the end of the sixth induction application, no further induction applications were administered to avoid mortality of the study animals. The initial dose mean irritation score for the test article group was zero and the group mean irritation score for all six induction applications was 0.04. The mean irritation score at the challenge dose was 0.25. The large increase in irritation scores at the challenge dose compared to the induction phase is also indicative of a positive sensitization response.

A sham control group of ten animals received the initial application of two 0.1 ml intradermal injections of Freund's complete adjuvant without being

induced with the six induction applications of the test material. The sham control group received the same dose, exposure and observations as the test article group during the challenge phase of the study. There was no dermal irritation evident in all sham control animals at the challenge dose evaluation. The results indicate the administration of adjuvant did not influence the degree of irritation noted in the test article group at the challenge dose evaluation.

A positive control group of ten animals using 2,4-Dinitrochlorobenezene, was induced in the same manner as the test article group. Subsequent challenge of the positive control group showed extreme sensitization with 10/10 animals showing a positive response. The initial dose mean irritation score for the positive control group was zero and the mean irritation score for all six induction applications was 0.38. The mean irritation score for the positive control group at the challenge dose was 1.70. The large increase in irritation at the challenge dose when compared to the induction phase is also indicative of a positive sensitization response in the positive control group.

Introduction

The object of this study was to determine the sensitization potential of hydroxypropylmethylcellulose gel containing 50% pyridostigmine bromide in female albino guinea pigs. This study was conducted in accordance with the Food and Drug Administration's Good Laboratory Practice Regulation of 1978. The raw data generated by the Study Director and the final report are stored in the conducting laboratory's archives.

Method and Results

Thirty albino guinea pigs of the Hartley strain[a] were used to evaluate the sensitization potential of the hydroxypropylmethylcellulose gel containing 50% pyridostigmine bromide. All animals were held under quarantine for several days prior to testing and only animals which appeared to be in good health and suitable as test animals at the initiation of the study were used. The guinea pigs were housed in temperature and humidity controlled rooms and permitted a standard laboratory diet[b] and water ad libitum. The test method was a modification of Buehler[c].

The induction phase consisted of six topical applications of the test article[d] on the dorsal shoulder girdle of ten animals, which had been clipped free of hair prior to the application. The initial topical application also included two 0.1 ml intradermal injections of Freund's Adjuvant[e] (1:1 commercial adjuvant with water) close to the area where the test article was immediately applied. The patches were secured with gauze and this in turn was firmly secured with elastic bandage material[f]. The patches were left in place for an approximately 24 hour contact period after which the patches and all residual test article were removed. Each application was then scored on a scale of 0 to 4 for erythema and edema[g] (Table 1) at approximately 24 hours after each test article removel. Six topical applications of the test article at three applications per week (Monday, Wednesday and Friday) were applied to the dorsal shoulder girdle of the ten test guinea pigs. Fourteen days after the final application,

[a] Charles River Breeding Laboratories, Inc., Wilmington, MA
[b] Ralston Purina Guinea Pig Chow, Ralston Purina, St. Louis, MO
[c] Buehler, EV: Delayed Contact Hypersensitivity in the Guinea Pig. Arch. Dermat. 91:171 (1965)
[d] Approximately 0.1 ml dose for each application
[e] Difco Labs, Inc., Detroit, MI
[f] Elastoplast , Beiersdorf, Inc., South Norwalk, CT

the hair was removed from an area on the flank (posterior to the induction site) and the test article applied in the same fashion as for the induction phase. This was left in place for approximately 24 hours. The challenge sites were evaluated one day and two days after removal of the patches, on a scale of 0 to 4 for erythema and edema.

The positive control (DNCB)[h] group consisting of ten animals was treated in the same manner as the test article group, using 0.1 ml of 0.1% DNCB in propylene glycol for induction and 0.05% for the challenge. A sham control group of ten animals received the initial application of two 0.1 ml intradermal injections of Freund's complete adjuvant without being induced with the six induction applications of the test material. The sham control group received the same dose, exposure and observations as the test article group during the challenge dose phase of the study. The results of the study are shown in Tables 2-4. The following grading system was used to arrive at a descriptive rating:

Sensitization Rate (%)	Classification
0 - 10	Weak
20 - 30	Mild
40 - 60	Moderate
70 - 80	Strong
90 - 100	Extreme

The protocol, principal personnel involved in the study, composition characteristics and Quality Assurance statement are contained in Appendices I-IV.

[h] 2,4-Dinitrochlorobenzene, Kodak, Lot A11G

TABLE 1

SENSITIZATION TEST - ALBINO GUINEA PIGS

Scoring Criteria for Skin Reactions

Reaction	Description	Score
Erythema	Barely perceptible (Edges of area not defined)	1
	Pale red in color and area definable	2
	Definite red in color and area well defined.	3
	Beet or crimson red in color	4
Edema	Barely perceptible (Edges of area not defined)	1
	Area definable but not raised more 1 mm.	2
	Area well defined and raised approximately 1 mm.	3
	Area raised more than 1 mm.	4
	Maximum Primary Irritation Score	= 8

TABLE 2

Guinea Pig Skin Sensitization Study With Hydroxypropylmethylcellulose Gel Containing 50% Pyridostigmine Bromide (Test Article Group)
Results

Animal #	Dose # / Hour	1 24	2 24	3 24	4 24	5 24	6 24	6 Dose Average	Challenge Dose 24	Challenge Dose 48
7G869**	Erythema	0	0	0	1	0	1		1	1
	Edema	0	0	0	0	0	0		0	1
	Average	0	0	0	0.50	0.00	0.50	0.17		0.75
7G875	Erythema	0	0	0	0	0			0	0
	Edema	0	0	0	0	0			0	0
	Average	0	0	0	0.00	0.00	0.00	0.00		0.00
7G881	Erythema	0	0	0	0	0			0	0
	Edema	0	0	0	0	0			0	0
	Average	0	0	0	0.00	0.00	0.00	0.00		0.00
7G887	Erythema	0	0	0	0	0	0		0	0
	Edema	0	0	0	0	0	0		0	0
	Average	0	0	0	0.00	0.00	0.00	0.00		0.00
7G893	Erythema	0	0	0	0	0	0		1	1
	Edema	0	0	0	0	0	0		0	0
	Average	0	0	0	0.00	0.00	0.00	0.00		0.50
7G870	Erythema	0	0	0	0	0	1		1	1
	Edema	0	0	0	0	0	0		0	0
	Average	0	0	0	0.00	0.00	0.50	0.08		0.50
7G876	Erythema	0	0	0	0	0	0		0	0
	Edema	0	0	0	0	0	0		0	0
	Average	0	0	0	0.00	0.00	0.00	0.00		0.00
7G882	Erythema	0	0	0	0	0	0		0	0
	Edema	0	0	0	0	0	0		0	0
	Average	0	0	0	0.00	0.00	0.00	0.00		0.00
7G888*	Erythema	0	0	0	0	0	0			
	Edema	0	0	0	0	0	0			
	Average	0	0	0	0.00	0.00	0.00	0.00		---
7G894	Erythema	0	0	0	0	0	1		1	1
	Edema	0	0	0	0	0	0		0	0
	Average	0	0	0	0.00	0.00	0.50	0.08		0.50

Group, Initial Dose Average = 0

Group, 6 Dose Average = 0.04

Group, Challenge Dose Average = 0.25

* = Animal found dead

** = Tremors and salivation noted approximately one hour after dose administration of Dose # 6.

4/9 animals positive - 44% Sensitization Rate = Grade III or moderate sensitizer.

Note: The values for animals found dead during the study are not used in the group average calculations.

TABLE 3

Guinea Pig Skin Sensitization Study With Hydroxypropylmethylcellulose Gel Containing 50% Pyridostigmine Bromide (Sham Control Group) Results

Animal #	Hour	Challenge Dose 24	48
7G899	Erythema	0	0
	Edema	0	0
	Average	0	
7G905	Erythema	0	0
	Edema	0	0
	Average	0	
7G911	Erythema	0	0
	Edema	0	0
	Average	0	
7G917	Erythema	0	0
	Edema	0	0
	Average	0	
7G923	Erythema	0	0
	Edema	0	0
	Average	0	
7G900	Erythema	0	0
	Edema	0	0
	Average	0	
7G906	Erythema	0	0
	Edema	0	0
	Average	0	
7G912	Erythema	0	0
	Edema	0	0
	Average	0	
7G918	Erythema	0	0
	Edema	0	0
	Average	0	
7G924	Erythema	0	0
	Edema	0	0
	Average	0	

Group Challenge Dose Average = 0

TABLE 4

Guinea Pig Skin Sensitization Study With DNCB (Positive Control Group)
Results

Animal #	Dose # / Hour	1 24	2 24	3 24	4 24	5 24	6 24	6 Dose Average	Challenge Dose 24	Challenge Dose 48
7G929	Erythema	0	0	0	1	1	3		2	2
	Edema	0	0	0	0	0	2		1	1
	Average	0	0	0	0.50	0.50	2.50	0.58	1.5	
7G935	Erythema	0	0	0	0	1	1		2	2
	Edema	0	0	0	0	0	0		2	2
	Average	0	0	0	0.00	0.50	0.50	0.17	2.0	
7G941	Erythema	0	0	0	2	2	3		3	3
	Edema	0	0	0	1	1	2		2	2
	Average	0	0	0	1.50	1.50	2.50	0.92	2.50	
7G947	Erythema	0	0	0	0	1	1		2	3
	Edema	0	0	0	0	0	0		2	2
	Average	0	0	0	0.00	0.50	0.50	0.17	2.25	
7G950	Erythema	0	0	0	0	1	1		2	2
	Edema	0	0	0	0	0	0		1	2
	Average	0	0	0	0.00	0.50	0.50	0.17	1.75	
7G930	Erythema	0	0	0	1	1	2		2	2
	Edema	0	0	0	1	1	1		1	1
	Average	0	0	0	1.00	1.00	1.50	0.58	1.50	
7G936	Erythema	0	0	0	0	0	0		1	1
	Edema	0	0	0	0	0	0		1	1
	Average	0	0	0	0.00	0.00	0.00	0.00	1.00	
7G942	Erythema	0	0	0	0	1	3		2	2
	Edema	0	0	0	0	0	2		1	1
	Average	0	0	0	0.00	0.50	2.50	0.50	1.50	
7G948	Erythema	0	0	0	1	2	2		2	2
	Edema	0	0	0	0	0	0		1	1
	Average	0	0	0	0.50	1.00	1.00	0.42	1.50	
7G945	Erythema	0	0	0	0	1	2		2	2
	Edema	0	0	0	0	0	1		1	1
	Average	0	0	0	0.00	0.50	1.50	0.33	1.50	

Group, Initial Dose Average = 0

Group, 6 Dose Average = 0.38

Group, Challenge Dose Average = 1.70

10/10 animals positive - 100% Sensitization Rate = Grade V or extreme sensitizer.

RIKER Experiment No.: 0387MG0051

APPENDIX I

PROTOCOL

[illegible] STUDY 9.

859

TEST: Modified Buehler[a] Sensitization Test

SPONSOR: 3M RIKER Division

CONDUCTED BY: **Pathology and Toxicology Department, Riker Laboratories, Inc., St. Paul, Minnesota**

TEST ARTICLE: Hydroxypropylmethylcellulose gel containing 50% w/w Pyridostigmine Bromide, Lot FN 4588

POSITIVE CONTROL ARTICLE: 2,4 - Dinitrochlorobenzene

PROPOSED STARTING/COMPLETION DATE OF TEST: 3/87 - 7/87

TEST SYSTEM: Hartley Strain, Guinea Pig (of either sex)

AGE: 6-10 weeks

SOURCE: Charles River Breeding Laboratories, Wilmington, Massachusetts

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OBJECTIVE: To determine the sensitization potential of the test article. Guinea pigs will be used as the test system due to their historical use and ease of handling.

METHOD: The method will be a modification of Buehler[a]. The animals will be housed in standard cages[b] in temperature and humidity controlled rooms with food and water offered ad libitum. The animals' numbers will be placed on cards affixed to the outside of their cages. The test will be conducted in two phases; an induction phase and a challenge phase. The induction phase will consist of nine topical applications of the test article[c] at three applications per week (Monday, Wednesday and Friday) in the dorsal shoulder girdle, which will be clipped free of hair prior to the application procedure. The initial procedure will consist of two injections of 0.1 ml (per injection - one/side) of Freund's Adjuvant[d] (1:1 commercial adjuvant with water) in each of the ten animals that will be administered the test article and in each of the ten animals that will be administered the positive control[e]. The test article will be placed near the injection sites and firmly secured. The test article will be left in place for approximately 24 hours, after which all residual test material will be removed. The subsequent applications will be done in the same manner as the initial application excluding the injection of adjuvant. The positive control group will be dosed in the same manner as the test article group. Each animal will be evaluated[f] for signs of skin irritation approximately 24 hours after removal of the test article for each of the nine exposures. Two weeks after the induction phase is complete, the hair will be removed from an area on the flank (posterior to the induction site). The test article will be applied to the site, secured and left in place for approximately 24 hours. The challenge sites will be evaluated approximately 24 and 48 hours after removal of all test articles. All skin reactions will be scored on the basis of 0 to 4[f]. An additional sham control group of ten animals will

[illegible] PATHOLOGY/TOXICOLOGY

PROTOCOL (continued)

METHOD: receive the initial application of two injections of 0.1 ml of Freund's adjuvant without being induced with the nine induction applications of the test material. The sham control group will receive the same dose, exposure and observations as the test article group during the challenge dose phase of the study. The sham control group and the test article group will be utilized to compare any differences between the two groups in severity of skin irritation present at the challenge dose site. The comparison should allow the study director to separate irritation caused by the test material that may be present only because the animals received adjuvant alone from irritation that is indicative of sensitization due to the nine induction applications. All raw data generated by the study director and the final report will be stored in Riker Laboratories' Archives, St. Paul, Minnesota.

a - Buehler, EV; Delayed Contact Hypersensitivity in the Guinea Pig, Arch. Dermat. 91:171 (1965)
b - Purina Guinea Pig Chow, Ralston Purina Co., St. Louis, Missouri
c - The test article dose will be 0.1 ml
d - Difco Laboratories, Inc., Detroit, Michigan
e - 0.1 ml of 2,4-dinitrochlorobenzene, Sigma Chemical Co., St. Louis, Missouri
f - Draize: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965)

Marie Wolfe 2/27/87
Sponsor Date

Gene L. Harris 3/2/87
Study Director Date

Riker Experiment No. 0387MG0051

861

Appendix I (concluded)
Deviations and/or Amendments to Protocol

1. The induction phase will be limited to 6 doses. Reason for change: to avoid mortality in the study animals due to test material.

Gene L. Harris — 3/17/87
Study Director — Date

2. Pyridostigmine bromide may also be named "S-26741" in this study. Reason for change: S-26741 is the Riker name given to pyridostigmine bromide.

Gene L. Harris — 4/16/87
Study Director — Date

3.

Study Director — Date

4.

Study Director — Date

APPENDIX II

Principal Participating Personnel Involved in the Study

Name	Function
G. L. Harris, B.S.	Advanced Toxicologist Study Director
G. E. Hart	Master Laboratory Technician Acute Toxicology
L. O. Wiseth	Sr. Laboratory Technician Acute Toxicology
J. A. Eads	Jr. Laboratory Technician Acute Toxicology
J. L. Allen, Ph.D.	Research Toxicologist Acute Toxicology
G. C. Pecore	Supervisor Animal Laboratory

APPENDIX III

863

Test and/or Control Article Characterization

for

Hydroxypropylmethylcellulose gel Containing 50% w/w pyridostigmine Bromide (FN 4588)

1. The identity strength, uniformity, composition, purity or other pertinent characterizations of the test and/or substances have been determined and documented as of RFA 14203 -2/27/87.

2. The method of synthesis or origin of the test and control substances, including their amount and the method of bioassay (if applicable) is documented.

☐ Yes ☐ No Analytical Dir. 2/27/87

3. The stability of the test and/or control substances ~~have been determined or~~ will be determined ~~as of~~* to end of GLP study.
AT Analytical Dir. (2/27/87)

The above information and documentation are located in the sponsor's records.

~~Sponsor or~~ Sponsor Representative	Date
Analytical Dir.	2/27/87

* = Form change

Fox 19793-A-PWO

APPENDIX IV

QUALITY ASSURANCE STATEMENT

Acute Toxicology Laboratory Studies

Study No.: 0387M60051

This short term study was audited by Compliance Audit and the final report examined against the raw data on April 21, 1987. The results of the audit were reported to the study director and to management on April 21, 1987.

In addition to the data audit, different significant phases for studies underway in the Acute Toxicology Laboratory are inspected on a recurring cycle, and the facilities are examined by Compliance Audit on a three month schedule.

D. M. Markow

Compliance Audit

4-21-87

Date

Pathology and Toxicology
Riker Laboratories, Inc.

Building 270-3S-05, 3M Center
St. Paul, Minnesota 55144-1000

COMPANY CONFIDENTIAL

3M

Sensitizaton Study

with Hydroxypropylmethylcellulose Gel Containing 50%

Pyridostigmine Bromide, Lot # FN4588

in Albino Guinea Pigs

Riker Experiment No: 0387MG0052

Conducted At: Pathology and Toxicology
Riker Laboratories, Inc.

Dates Conducted: March 4, 1987 to April 2, 1987

Conducted By: Gene L. Harris 4/22/87

G. L. Harris, B.S. Date
Advanced Toxicologist
Study Director and Acute Toxicity
Study Coordinator

Reviewed By: J Allen 4-22-87

J. L. Allen, Ph.D. Date
Diplomate, A.B.T.
Research Toxicologist

dc: R.T. Catherall
M.W. Downing
N.M. Marecki
M.J. Westfall (2)
Tech. Doc. Center
Path/Tox Files

Summary

A skin sensitization study was conducted from March 4, 1987 to April 2, 1987, at Riker Laboratories, Inc., St. Paul, Minnesota. The results indicate that Hydroxypropylmethylcellulose gel containing 50% pyridostigmine bromide is a potentially moderate sensitizer with positive responses noted in 4/9 animals. An individual animal was considered positive in this test if irritation was noted in the animal at the challenge dose evaluation. The initial induction dose application score for each animal in this study was zero.

Ten female albino guinea pigs in the test article group received six topical induction applications of the potential antigen and subsequently challenged topically 14 days post induction. One of the animals (7G864) appeared pregnant during the second week of the study and, therefore, is not included in the final evaluation, although the irritation scores for this animal are listed in Table 2. Salivation and tremors were noted in one animal (7G852) at one hour after dose administration of the sixth induction application. Due to the adverse effects in test article-treated animals by the end of the sixth induction application, no further induction applications were administered to avoid mortality. The initial dose mean irritation score for the test article group was zero and the mean irritation score for all six induction applications was 0.03. The mean irritation score for the test article group at the challenge dose was 0.22. The large increase in irritation scores for the challenge dose compared to the induction phase is also indicative of a positive sensitization response.

A positive control group of ten animals using 2,4-Dinitrochlorobenzene, was induced in the same manner as the test article group. Subsequent challenge of the positive control group showed extreme sensitization with 10/10 animals showing a positive response. The initial dose mean irritation score for the positive control group was zero and the mean irritation score for all six induction applications was 0.23. The mean irritation score for the positive control group at the challenge dose was 1.33. The large increase in irritation noted at the challenge dose when compared to the induction phase is also indicative of a positive sensitization response in the positive control group.

Introduction

The object of this study was to determine the sensitization potential of hydroxypropylmethylcellulose gel containing 50% pyridostigmine bromide, Lot # FN4588, in female albino guinea pigs. This study was conducted in accordance with the Food and Drug Administration's Good Laboratory Practice Regulation of 1978. The raw data generated by the Study Director and the final report are stored in the conducting laboratory's archives.

Method and Results

Twenty albino guinea pigs of the Hartley strain[a] were used to evaluate the sensitization potential of hydroxypropylmethylcellulose gel containing pyridostigmine bromide, Lot # FN4588. All animals were held under quarantine for several days prior to testing and only animals which appeared to be in good health and suitable as test animals at the initiation of the study were used. The guinea pigs were housed in temperature and humidity controlled rooms and permitted a standard laboratory diet[b] and water ad libitum. The test method was a modification of Buehler[c].

The induction phase consisted of six topical applications of the test article[d] on the dorsal shoulder girdle of ten animals, which had been clipped free of hair prior to the application. The test article was secured with gauze and this in turn was firmly secured with elastic bandage material[f]. The patches were left in place for approximately a 24 hour contact period after which the patches and all residual test article were removed. Each application was then scored on a scale of 0 to 4 for erythema and edema[g] (Table 1) at approximately 24 hours after each test article removal. Six topical applications of the test article at three applications per week (Monday, Wednesday and Friday) were applied to the dorsal shoulder girdle of the ten guinea pigs. Fourteen days after the

[a] Charles River Breeding Laboratories, Inc., Wilmington, MA
[b] Ralston Purina Guinea Pig Chow, Ralston Purina, St. Louis, MO
[c] Buehler, EV: Delayed Contact Hypersensitivity in the Guinea Pig. Arch. Dermat. 91:171 (1965)
[d] 0.1 ml dose for each application
[e] Difco Labs, Inc., Detroit, MI
[f] Elastoplast , Beiersdorf, Inc., South Norwalk, CT
[g] Draize: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965).

final induction application, the hair was removed from an area on the flank (posterior to the induction site) and the test article was applied in the same fashion as in the induction phase. This was left in place for approximately 24 hours. The challenge sites were evaluated one day and two days after removal of the patches, on a scale of 0 to 4 for erythema and edema.

The positive control (DNCB) group consisting of ten animals was treated in the same manner as the test article group, using 0.1 ml of 0.1% DNCB in propylene glycol for induction and 0.05% for the challenge. The results of the study are shown in Tables 2 and 3. The following grading system was used to arrive at a descriptive rating:

Sensitization Rate (%)	Classification
0 - 10	Weak
20 - 30	Mild
40 - 60	Moderate
70 - 80	Strong
90 - 100	Extreme

The protocol, principal personnel involved in the study, composition characteristics and Quality Assurance statement are contained in Appendices I-IV.

TABLE 1

SENSITIZATION TEST - ALBINO GUINEA PIGS

Scoring Criteria for Skin Reactions

Reaction	Description	Score
Erythema	Barely perceptible (Edges of area not defined)	1
	Pale red in color and area definable	2
	Definite red in color and area well defined.	3
	Beet or crimson red in color	4
Edema	Barely perceptible (Edges of area not defined)	1
	Area definable but not raised more 1 mm.	2
	Area well defined and raised approximately 1 mm.	3
	Area raised more than 1 mm.	4
	Maximum Primary Irritation Score	= 8

TABLE 2

Sensitization Study With Hydroxypropylmethylcellulose Gel Containing 50% Pyridostigmine Bromide in Albino Guinea Pigs
Results

Animal #	Dose # / Hour	1 24	2 24	3 24	4 24	5 24	6 24	6 Dose Average	Challenge Dose 24	Challenge Dose 48
7G839	Erythema	0	0	0	0	0	0		1	1
	Edema	0	0	0	0	0	0		0	0
	Average	0	0	0	0	0	0	0	0.5	
7G845	Erythema	0	0	0	0	0	1		1	1
	Edema	0	0	0	0	0	0		0	0
	Average	0	0	0	0	0	0.50	0.08	0.5	
7G851	Erythema	0	0	0	0	0	0		0	0
	Edema	0	0	0	0	0	0		0	0
	Average	0	0	0	0	0	0	0	0.0	
7G857	Erythema	0	0	0	0	0	0		1	1
	Edema	0	0	0	0	0	0		0	0
	Average	0	0	0	0	0	0	0	0.0	
7G863	Erythema	0	0	0	0	0	0		0	0
	Edema	0	0	0	0	0	0		0	0
	Average	0	0	0	0	0	0	0	0.0	
7G840	Erythema	0	0	0	0	0	0		0	0
	Edema	0	0	0	0	0	0		0	0
	Average	0	0	0	0	0	0	0	0.0	
7G846	Erythema	0	0	0	0	0	0		0	0
	Edema	0	0	0	0	0	0		0	0
	Average	0	0	0	0	0	0	0	0.0	
7G852*	Erythema	0	0	0	0	0	1		0	0
	Edema	0	0	0	0	0	1		0	0
	Average	0	0	0	0	0	1.0	0.17	0.0	
7G858	Erythema	0	0	0	0	0	0		1	1
	Edema	0	0	0	0	0	0		0	0
	Average	0	0	0	0	0	0	0	0.5	
7G864**	Erythema	0	0	0	0	0	0		0	0
	Edema	0	0	0	0	0	0		0	0
	Average	0	0	0	0	0	0	0	0.0	

Group, Initial Dose Average = 0

Group, 6 Dose Average = 0.03

Group, Challenge Dose Average = 0.22

* = Salivation and tremors noted at 1 hour after administration of dose #6.
** = Animal noted as being pregnant during second week of the study.

4/9 animals positive - 44% Sensitization Rate = Grade III or moderate sensitizer.

Note: The values for the pregnant guinea pig are not included in the group average calculations.

TABLE 3

Sensitization Study With DNCB (Positive Control)
in Albino Guinea Pigs
Results

Animal #	Dose # / Hour	1 24	2 24	3 24	4 24	5 24	6 24	6 Dose Average	Challenge Dose 24	Challenge Dose 48
7G931	Erythema	0	0	0	0	1	1		2	2
	Edema	0	0	0	0	0	0		1	1
	Average	0	0	0	0	0.50	0.50	0.17	1.5	
7G937	Erythema	0	0	0	1	1	1		2	2
	Edema	0	0	0	0	0	0		1	1
	Average	0	0	0	0.50	0.50	0.50	0.25	1.5	
7G943	Erythema	0	0	0	0	1	1		1	1
	Edema	0	0	0	0	0	0		2	1
	Average	0	0	0	0	0.50	0.50	0.17	1.25	
7G949	Erythema	0	0	0	1	1	3		2	2
	Edema	0	0	0	0	1	2		1	1
	Average	0	0	0	0.50	1.00	2.50	0.67	1.5	
7G946	Erythema	0	0	0	0	0	1		1	1
	Edema	0	0	0	0	0	0		1	1
	Average	0	0	0	0	0	0.50	0.08	1.00	
7G932	Erythema	0	0	0	0	0	0		1	1
	Edema	0	0	0	0	0	0		1	1
	Average	0	0	0	0	0	0	0	1.00	
7G938	Erythema	0	0	0	0	0	0		1	1
	Edema	0	0	0	0	0	0		1	1
	Average	0	0	0	0	0	0	0	1.00	
7G944	Erythema	0	0	0	1	1	3		2	2
	Edema	0	0	0	0	0	2		2	2
	Average	0	0	0	0.50	0.50	2.50	0.58	2.00	
7G940	Erythema	0	0	0	0	0	2		2	2
	Edema	0	0	0	0	0	1		1	1
	Average	0	0	0	0	0	1.50	0.25	1.5	
7G934	Erythema	0	0	0	0	0	1		1	1
	Edema	0	0	0	0	0	0		1	1
	Average	0	0	0	0	0	0.50	0.08	1.00	

Group, Initial Dose Average = 0

Group, 6 Dose Average = 0.23

Group, Challenge Dose Average = 1.33

10/10 animals positive - 100% Sensitization Rate = Grade V or extreme sensitizer.

APPENDIX I

PROTOCOL

873 GLP STUDY 8.

TEST: Modified Buehler[a] Sensitization Test

SPONSOR: 3M RIKER Division

CONDUCTED BY: **Pathology and Toxicology Department, Riker Laboratories, Inc., St. Paul, Minnesota**

TEST ARTICLE: Hydroxypropylmethylcellulose gel containing 50% w/w Pyridostigmine Bromide Lot # FN4588

POSITIVE CONTROL ARTICLE: 2,4 - Dinitrochlorobenzene

PROPOSED STARTING/COMPLETION DATE OF TEST: 3/87 - 7/87

TEST SYSTEM: Hartley Strain, Guinea Pig (of either sex)

AGE: 6-10 weeks

SOURCE: Charles River Breeding Laboratories, Wilmington, Massachusetts

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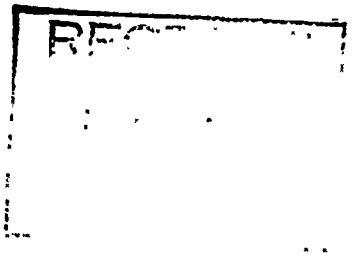
OBJECTIVE: To determine the sensitization potential of the test article. Guinea pigs will be used as the test system due to their historical use and ease of handling.

METHOD: The method will be a modification of Buehler[a]. The animals will be housed in standard[b] cages in temperature and humidity controlled rooms with food and water offered **ad libitum**. The animals' numbers will be placed on cards affixed to the outside of their cages. The test will be conducted in two phases; an induction phase and a challenge phase. The induction phase will consist of nine topical applications of the test article[c] at three applications per week (Monday, Wednesday and Friday) in the dorsal shoulder girdle, which will be clipped free of hair prior to the application procedure. A group of ten animals will be administered the test article and an additional group of ten animals will be administered the positive control[d]. The test article will be applied and firmly secured. The test article will be left in place for approximately 24 hours, after which all residual test material will be removed. The subsequent applications will be done in the same manner as the initial application. The positive control group will be dosed in the same manner as the test article group. Each animal will be evaluated[e] for signs of skin irritation approximately 24 hours after removal of the test article for each of the nine exposures. Two weeks after the induction phase is complete, the hair will be removed from an area on the flank (posterior to the induction site). The test article will be applied to the site,



METHOD: secured and left in place for approximately 24 hours. The challenge sites will be evaluated approximately 24 and 48 hours after removal of all test articles. All skin reactions will be scored on the basis of 0 to 4[a].

[a] Buehler, EV; Delayed Contact Hypersensitivity in the Guinea Pig, Arch. Dermat. 91:171 (1965)
[b] Purina Guinea Pig Chow, Ralston Purina Co., St. Louis, Missouri
[c] The test article dose will be 0.1 ml
[d] 0.1 ml of 2,4-dinitrochlorobenzene, Sigma Chemical Co., St. Louis, Missouri
[e] Draize: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965)

Marie Wolfall 2/27/87
Sponsor Date

Gene L. Harris 3/2/87
Study Director Date

Riker Experiment No. 0387MG0052

Appendix I (concluded)
Deviations and/or Amendments to Protocol

1. The induction phase will be limited to 6 doses. Reason for change: to avoid mortality in the study animals due to test material.

Gene L. Harris — 3/17/87
Study Director — Date

2. Pyridostigmine bromide may also be named by S-26741 in the raw data of this study. Reason for change: two different names for the same thing, S-26741 in the Riker # given to pyridostigmine bromide.

Gene L. Harris — 4/13/87
Study Director — Date

3.

Study Director — Date

4.

Study Director — Date

APPENDIX II

Principal Participating Personnel Involved in the Study

Name	Function
G. L. Harris, B.S.	Advanced Toxicologist Study Director
G. E. Hart	Master Laboratory Technician Acute Toxicology
L. O. Wiseth	Sr. Laboratory Technician Acute Toxicology
J. A. Eads	Jr. Laboratory Technician Acute Toxicology
J. L. Allen, Ph.D.	Research Toxicologist Acute Toxicology
G. C. Pecore	Supervisor Animal Laboratory

APPENDIX III

Test and/or Control Article Characterization

for

Hydroxypropylmethylcellulose gel containing 50% w/w pyridostigmine Bromide (FN 4588)

1. The identity strength, uniformity, composition, purity or other pertinent characterizations of the test and/or substances have been determined and documented as of RFA 14203 - 2/27/87.

2. The method of synthesis or origin of the test and control substances, including their amount and the method of bioassay (if applicable) is documented.

   ☐ Yes ☐ No Amitech Inc. to 2/27/87 *

3. The stability of the test and/or control substances ~~have been determined or~~ will be determined ~~as of~~* the end of the study. AT Amitech Inc (2/27/87)

The above information and documentation are located in the sponsor's records.

~~Sponsor or~~ Sponsor Representative	Date
Amitech Inc.	2/27/87

Original Characterization can be found in experiment No. 0387MG0651.

* = Form CHANGE

APPENDIX IV

QUALITY ASSURANCE STATEMENT

Acute Toxicology Laboratory Studies

Study No.: 0387M60052

This short term study was audited by Compliance Audit and the final report examined against the raw data on April 21, 1987. The results of the audit were reported to the study director and to management on April 21, 1987.

In addition to the data audit, different significant phases for studies underway in the Acute Toxicology Laboratory are inspected on a recurring cycle, and the facilities are examined by Compliance Audit on a three month schedule.

D. M. Warboys

Compliance Audit

4-21-87

Date

Pathology and Toxicology
Riker Laboratories, Inc.

Building 270-3S-05, 3M Center
St. Paul, Minnesota 55144-1000

COMPANY CONFIDENTIAL

3M

Sensitizaton Study

with Hydroxypropylmethylcellulose Gel Containing 30%

Pyridostigmine Bromide and 0.21% Docusate Sodium

in Albino Guinea Pigs

Riker Experiment No: 0387MG0054

Conducted At: Pathology and Toxicology
Riker Laboratories, Inc.

Dates Conducted: March 4, 1987 to April 2, 1987

Conducted By: Gene L. Harris 4/22/87

G. L. Harris, B.S. Date
Advanced Toxicologist
Study Director and Acute Toxicity
Study Coordinator

Reviewed By: J Allen 4-22-87

J. L. Allen, Ph.D. Date
Diplomate, A.B.T.
Research Toxicologist

dc: R.T. Catherall
M.W. Downing
N.M. Marecki
[redacted]
Tech. Doc. Center
Path/Tox File

Summary

A skin sensitization study was conducted from March 4, 1987 to April 2, 1987 at Riker Laboratories, Inc., St. Paul, Minnesota. The results indicate that hydroxypropylmethylcellulose gel containing 30% pyridostigmine bromide and 0.21% docusate sodium is a potentially extreme sensitizer with positive responses noted in 7/8 animals. An individual animal was considered positive in this test if irritation was noted in the animal at the challenge dose evaluation. The initial induction dose application score for each animal in this study was zero.

All animals in all groups in this study received two, 0.1 ml intradermal injections of Freund's complete adjuvant just prior to the initial induction dose.

Ten female albino guinea pigs in the test article group received six topical induction applications of the potential antigen and subsequently challenged topically 14 days post induction. Two animals in the test article group were found dead just after dose administration of the sixth induction application. Tremors and salivation were noted in these animals prior to death. The two animals found dead are not included in the final evaluation, although the irritation scores for these animals are listed in Table 2. Due to the adverse effects noted in test article-treated animals by the end of the sixth inductin application, no further induction applications were administered to avoid additional mortality of the study animals. The initial dose mean irritatin score for the test article group was zero and the mean irritation score for all six induction applications was 0.30. The mean irritation score at the challenge dose was 1.06. The large increase in irritation scores at the

challenge dose compared to the induction phase is also indicative of a positive sensitization response.

A sham control group of ten animals received the initial application of two 0.1 ml intradermal injections of Freund's complete adjuvant without being induced with the six induction applications of the test material. The sham control group received the same dose, exposure and observations as the test article group during the challenge phase of the study. There was no dermal irritation evident in all sham control animals at the challenge dose evaluation. The results indicate the administration of adjuvant did not influence the degree of irritation noted in the test article group at the challenge dose evaluation.

A positive control group of ten animals using 2,4-Dinitrochlorobenezene, was induced in the same manner as the test article group. Subsequent challenge of the positive control group showed extreme sensitization with 10/10 animals showing a positive response. The group initial dose mean irritation score for the positive control group was zero and the group mean irritation score for all six induction applications was 0.38. The mean irritation score for the positive control group at the challenge dose was 1.70. The large increase in irritation noted at the challenge dose when compared to the induction phase is also indicative of a positive sensitization response in the positive control group.

Introduction

The object of this study was to determine the sensitization potential of hydroxypropylmethylcellulose gel containing 30% pyridostigmine bromide and 0.21% docusate sodium in female albino guinea pigs. This study was conducted in accordance with the Food and Drug Administration's Good Laboratory Practice Regulation of 1978. The raw data generated by the Study Director and the final report are stored in the conducting laboratory's archives.

Method and Results

Thirty albino guinea pigs of the Hartley strain[a] were used to evaluate the sensitization potential of the hydroxypropylmethylcellulose gel containing 30% pyridostigmine bromide and 0.21% docusate sodium. All animals were held under quarantine for several days prior to testing and only animals which appeared to be in good health and suitable as test animals at the initiation of the study were used. The guinea pigs were housed in temperature and humidity controlled rooms and permitted a standard laboratory diet[b] and water ad libitum. The test method was a modification of Buehler[c].

The induction phase consisted of six topical applications of the test article[d] on the dorsal shoulder girdle of ten animals, which had been clipped free of hair prior to the application. The initial topical application also included two 0.1 ml intradermal injections of Freund's Adjuvant[e] (1:1 commercial adjuvant with water) close to the area where the test article was immediately applied. The patches were secured with gauze and this in turn was firmly secured with elastic bandage material[f]. The patches were left in place for an approximately 24 hour contact period after which the patches and all residual test article were removed. Each application was then scored on a scale of 0 to 4 for erythema and edema[g] (Table 1) at approximately 24 hours after each test article removel. Six topical applications of the test article at three applications per week (Monday, Wednesday and Friday) were applied to the dorsal shoulder girdle of the ten test guinea pigs. Fourteen days after the final application,

[a] Charles River Breeding Laboratories, Inc., Wilmington, MA
[b] Ralston Purina Guinea Pig Chow, Ralston Purina, St. Louis, MO
[c] Buehler, EV: Delayed Contact Hypersensitivity in the Guinea Pig. Arch. Dermat. 91:171 (1965)
[d] 0.1 ml dose for each application
[e] Difco Labs, Inc., Detroit, MI
[f] Elastoplast , Beiersdorf, Inc., South Norwalk, CT

the hair was removed from an area on the flank (posterior to the induction site) and the test article applied in the same fashion as for the induction phase. This was left in place for approximately 24 hours. The challenge sites were evaluated one day and two days after removal of the patches, on a scale of 0 to 4 for erythema and edema.

The positive control (DNCB)[h] group consisting of ten animals was treated in the same manner as the test article group, using 0.1 ml of DNCB in propylene glycol for induction and for the challenge. A sham control group of ten animals received the initial application of two 0.1 ml intradermal injections of Freund's complete adjuvant without being induced with the six induction applications of the test material. The sham control group received the same dose, exposure and observations as the test article group during the challenge dose phase of the study. The results of the study are shown in Tables 2-4. The following grading system was used to arrive at a descriptive rating:

Sensitization Rate (%)	Classification
0 - 10	Weak
20 - 30	Mild
40 - 60	Moderate
70 - 80	Strong
90 - 100	Extreme

The protocol, principal personnel involved in the study, composition characteristics and Quality Assurance statement are contained in Appendices I-IV.

[h] 2,4-Dinitrochlorobenzene, Kodak, Lot A11G

TABLE 1

SENSITIZATION TEST - ALBINO GUINEA PIGS

Scoring Criteria for Skin Reactions

Reaction	Description	Score
Erythema	Barely perceptible (Edges of area not defined)	1
	Pale red in color and area definable	2
	Definite red in color and area well defined.	3
	Beet or crimson red in color	4
Edema	Barely perceptible (Edges of area not defined)	1
	Area definable but not raised more 1 mm.	2
	Area well defined and raised approximately 1 mm.	3
	Area raised more than 1 mm.	4
	Maximum Primary Irritation Score	= 8

TABLE 2

Guinea Pig Skin Sensitization Study With Hydroxypropylmethylcellulose Gel Containing 30% Pyridostigmine Bromide and 0.21% Docusate Sodium (Test Article Group)
Results

Animal #	Dose # / Hour	1 24	2 24	3 24	4 24	5 24	6 24	6 Dose Average	Challenge Dose 24	Challenge Dose 48
7G871	Erythema	0	0	0	1	1	2		1	1
	Edema	0	0	0	0	0	1		1	1
	Average	0	0	0	0.50	0.50	1.50	0.42	1.00	
7G877	Erythema	0	0	0	0	2				
	Edema	0	0	0	0	1				
	Average	0	0	0	0	1.50	X			
7G883	Erythema	0	0	0	1	2				
	Edema	0	0	0	0	1				
	Average	0	0	0	0.50	1.50	X			
7G889	Erythema	0	0	0	0	0	0		1	1
	Edema	0	0	0	0	0	0		1	1
	Average	0	0	0	0	0	0	0	1.00	
7G895	Erythema	0	0	0	1	0	1		2	2
	Edema	0	0	0	0	0	0		1	1
	Average	0	0	0	0.50	0	0.50	0.17	1.50	
7G872	Erythema	0	0	0	1	1	1		2	2
	Edema	0	0	0	1	1	1		1	1
	Average	0	0	0	1.00	1.00	1.00	0.5	1.50	
7G878	Erythema	0	0	0	0	1	2		2	2
	Edema	0	0	0	0	1	2		1	1
	Average	0	0	0	0	1.00	2.00	0.5	1.5	
7G884	Erythema	0	0	0	0	0	0		1	1
	Edema	0	0	0	0	0	0		1	1
	Average	0	0	0	0	0	0	0	1.00	
7G890	Erythema	0	0	0	1	1	2		0	0
	Edema	0	0	0	0	0	1		0	0
	Average	0	0	0	0.50	0.50	1.50	0.42	0.0	
7G896	Erythema	0	0	0	1	1	2		1	1
	Edema	0	0	0	0	0	1		1	1
	Average	0	0	0	0.50	0.50	1.50	0.42	1.00	

Group, Initial Dose Average = 0

Group, 6 Dose Average = 0.30

Group, Challenge Dose Average = 1.06

X = Animal found dead (salivation and tremors noted prior to death)

7/8 animals positive - 88% Sensitization Rate = Grade V or extreme sensitizer.

Note: The values for animals found dead during the study are not used in the group average calculations.

TABLE 3

Guinea Pig Skin Sensitization Study With Hydroxypropylmethylcellulose Gel Containing 30% Pyridostigmine Bromide and 0.21% Docusate Sodium (Sham Control Group)
Results

Animal #	Hour	Challenge Dose 24	48
7G901	Erythema	0	0
	Edema	0	0
	Average	0	
7G907	Erythema	0	0
	Edema	0	0
	Average	0	
7G913	Erythema	0	0
	Edema	0	0
	Average	0	
7G919	Erythema	0	0
	Edema	0	0
	Average	0	
7G925	Erythema	0	0
	Edema	0	0
	Average	0	
7G902	Erythema	0	0
	Edema	0	0
	Average	0	
7G908	Erythema	0	0
	Edema	0	0
	Average	0	
7G914	Erythema	0	0
	Edema	0	0
	Average	0	
7G920	Erythema	0	0
	Edema	0	0
	Average	0	
7G926	Erythema	0	0
	Edema	0	0
	Average	0	

Group Challenge Dose Average = 0

TABLE 4

Guinea Pig Skin Sensitization Study With DNCB (Positive Control Group)
Results

Animal #	Dose # / Hour	1 24	2 24	3 24	4 24	5 24	6 24	6 Dose Average	Challenge Dose 24	Challenge Dose 48
7G929	Erythema	0	0	0	1	1	3		2	2
	Edema	0	0	0	0	0	2		1	1
	Average	0	0	0	0.50	0.50	2.50	0.58	1.5	
7G935	Erythema	0	0	0	0	1	1		2	2
	Edema	0	0	0	0	0	0		2	2
	Average	0	0	0	0.00	0.50	0.50	0.17	2.0	
7G941	Erythema	0	0	0	2	2	3		3	3
	Edema	0	0	0	1	1	2		2	2
	Average	0	0	0	1.50	1.50	2.50	0.92	2.50	
7G947	Erythema	0	0	0	0	1	1		2	3
	Edema	0	0	0	0	0	0		2	2
	Average	0	0	0	0.00	0.50	0.50	0.17	2.25	
7G950	Erythema	0	0	0	0	1	1		2	2
	Edema	0	0	0	0	0	0		1	2
	Average	0	0	0	0.00	0.50	0.50	0.17	1.75	
7G930	Erythema	0	0	0	1	1	2		2	2
	Edema	0	0	0	1	1	1		1	1
	Average	0	0	0	1.00	1.00	1.50	0.58	1.50	
7G936	Erythema	0	0	0	0	0	0		1	1
	Edema	0	0	0	0	0	0		1	1
	Average	0	0	0	0.00	0.00	0.00	0.00	1.00	
7G942	Erythema	0	0	0	0	1	3		2	2
	Edema	0	0	0	0	0	2		1	1
	Average	0	0	0	0.00	0.50	2.50	0.50	1.50	
7G948	Erythema	0	0	0	1	2	2		2	2
	Edema	0	0	0	0	0	0		1	1
	Average	0	0	0	0.50	1.00	1.00	0.42	1.50	
7G945	Erythema	0	0	0	0	1	2		2	2
	Edema	0	0	0	0	0	1		1	1
	Average	0	0	0	0.00	0.50	1.50	0.33	1.50	

Group, Initial Dose Average = 0

Group, 6 Dose Average = 0.38

Group, Challenge Dose Average = 1.70

10/10 animals positive - 100% Sensitization Rate = Grade V or extreme sensitizer.

TEST: Modified Buehler[a] Sensitization Test

SPONSOR: 3M RIKER Division

CONDUCTED BY: **Pathology and Toxicology Department, Riker Laboratories, Inc., St. Paul, Minnesota**

TEST ARTICLE: HYDROXY PROPYL methylcellulose GEL containing 30% PYRIDOSTIGMINE BROMIDE AND 0.2% DOCUSATE SODIUM, Lot FN 4589

POSITIVE CONTROL ARTICLE: 2,4 - Dinitrochlorobenzene

PROPOSED STARTING/COMPLETION DATE OF TEST: 3/87 - 7/87

TEST SYSTEM: Hartley Strain, Guinea Pig (of either sex)

AGE: 6-10 weeks

SOURCE: Charles River Breeding Laboratories, Wilmington, Massachusetts

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OBJECTIVE: To determine the sensitization potential of the test article. Guinea pigs will be used as the test system due to their historical use and ease of handling.

METHOD: The method will be a modification of Buehler[a]. The animals will be housed in standard cages[b] in temperature and humidity controlled rooms with food and water offered ad libitum. The animals' numbers will be placed on cards affixed to the outside of their cages. The test will be conducted in two phases; an induction phase and a challenge phase. The induction phase will consist of nine topical applications of the test article[c] at three applications per week (Monday, Wednesday and Friday) in the dorsal shoulder girdle, which will be clipped free of hair prior to the application procedure. The initial procedure will consist of two injections of 0.1 ml (per injection - one/side) of Freund's Adjuvant[d] (1:1 commercial adjuvant with water) in each of the ten animals that will be administered the test article and in each of the ten animals that will be administered the positive control[e]. The test article will be placed near the injection sites and firmly secured. The test article will be left in place for approximately 24 hours, after which all residual test material will be removed. The subsequent applications will be done in the same manner as the initial application excluding the injection of adjuvant. The positive control group will be dosed in the same manner as the test article group. Each animal will be evaluated[f] for signs of skin irritation approximately 24 hours after removal of the test article for each of the nine exposures. Two weeks after the induction phase is complete, the hair will be removed from an area on the flank (posterior to the induction site). The test article will be applied to the site, secured and left in place for approximately 24 hours. The challenge sites will be evaluated approximately 24 and 48 hours after removal of all test articles. All skin reactions will be scored on the basis of 0 to 4[f]. An additional sham control group of ten animals will

889

PROTOCOL (continued)

METHOD: receive the initial application of two injections of 0.1 ml of Freund's adjuvant without being induced with the nine induction applications of the test material. The sham control group will receive the same dose, exposure and observations as the test article group during the challenge dose phase of the study. The sham control group and the test article group will be utilized to compare any differences between the two groups in severity of skin irritation present at the challenge dose site. The comparison should allow the study director to separate irritation caused by the test material that may be present only because the animals received adjuvant alone from irritation that is indicative of sensitization due to the nine induction applications. All raw data generated by the study director and the final report will be stored in Riker Laboratories' Archives, St. Paul, Minnesota.

[a] Buehler, EV; Delayed Contact Hypersensitivity in the Guinea Pig, Arch. Dermat. 91:171 (1965)
[b] Purina Guinea Pig Chow, Ralston Purina Co., St. Louis, Missouri
[c] The test article dose will be 0.1 ml
[d] Difco Laboratories, Inc., Detroit, Michigan
[e] 0.1 ml of 2,4-dinitrochlorobenzene, Sigma Chemical Co., St. Louis, Missouri
[f] Draize: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965)

Maria Lus Faes 2/27/87 Gene L. Harris 3/3/87
Sponsor Date Study Director Date

PA[illegible]

Riker Experiment No. 0387MG0054

890

Appendix I (concluded)
Deviations and/or Amendments to Protocol

1. The induction phase will be limited to 6 doses. Reason for change: to avoid mortality in the study animals due to test material.

Gene L. Harris — Study Director — 3/17/87 — Date

2. Pyridostigmine bromide may also be named "S-26741" in the raw data of this study. Reason for change: S-26741 is the Riker number given to pyridostigmine bromide.

Gene L. Harris — Study Director — 4/16/87 — Date

3.

Study Director — Date

4.

Study Director — Date

APPENDIX II

Principal Participating Personnel Involved in the Study

Name	Function
G. L. Harris, B.S.	Advanced Toxicologist Study Director
G. E. Hart	Master Laboratory Technician Acute Toxicology
L. O. Wiseth	Sr. Laboratory Technician Acute Toxicology
J. A. Eads	Jr. Laboratory Technician Acute Toxicology
J. L. Allen, Ph.D.	Research Toxicologist Acute Toxicology
G. C. Pecore	Supervisor Animal Laboratory

APPENDIX III

Test and/or Control Article Characterization

for

Hydroxypropylmethylcellulose gel containing 30% pyridostigmine Bromide and 0.2% Docusate Sodium, (FN 4589)

1. The identity strength, uniformity, composition, purity or other pertinent characterizations of the test and/or substances have been determined and documented as of RFA 14201 – 2/27/87.

2. The method of synthesis or origin of the test and control substances, including their amount and the method of bioassay (if applicable) is documented.

   ☐ Yes ☐ No Anita Tilton 2/27/87

3. The stability of the test and/or control substances* ~~have been determined or~~ will be determined ~~as of~~ AT the end of the study.

   Anita Tilton 2/27/87

The above information and documentation are located in the sponsor's records.

~~Sponsor or~~ Sponsor Representative	Date
Anita Tilton	2/27/87

* = Form change

APPENDIX IV

QUALITY ASSURANCE STATEMENT

Acute Toxicology Laboratory Studies

Study No.: 0387MG0054

This short term study was audited by Compliance Audit and the final report examined against the raw data on April 21, 1987. The results of the audit were reported to the study director and to management on April 21, 1987.

In addition to the data audit, different significant phases for studies underway in the Acute Toxicology Laboratory are inspected on a recurring cycle, and the facilities are examined by Compliance Audit on a three month schedule.

D.M. Marbaugh
Compliance Audit

4-21-87
Date

Pathology and Toxicology
Riker Laboratories, Inc.

Building 270-3S-05, 3M Center
St. Paul, Minnesota 55144-1000

COMPANY CONFIDENTIAL

3M

Sensitizaton Study with Hydroxypropylmethylcellulose Gel Containing 30% Pyridostigmine Bromide and 0.21% Docusate Sodium in Albino Guinea Pigs

Riker Experiment No: 0387MG0055

Conducted At: Pathology and Toxicology
Riker Laboratories, Inc.

Dates Conducted: March 4, 1987 to April 2, 1987

Conducted By: Gene L. Harris 4/22/87
G. L. Harris, B.S. Date
Advanced Toxicologist
Study Director and Acute Toxicity
Study Coordinator

Reviewed By: J Allen 4-22-87
J. L. Allen, Ph.D. Date
Diplomate, A.B.T.
Research Toxicologist

dc: R. T. Catherall
M. W. Downing
N. M. Marecki
M. J. Westfall (2)
Tech. Doc. Center
Path/Tox File

Summary

A skin sensitization study was conducted from March 4, 1987 to April 2, 1987 at Riker Laboratories, Inc., St. Paul, Minnesota. The results indicate that hydroxypropylmethylcellulose gel containing 30% pyridostigmine and 0.21% docusate sodium is a potentially strong sensitizer with positive reponses noted in 5/7 animals. An individual animal was considered positive in this test if irritation was noted in the animal at the challenge dose evaluation. The initial induction dose application score for each animal in this study was zero.

Ten female albino guinea pigs in the test article group received six topical induction applications of the potential antigen and subsequently challenged topically 14 days post induction. Three animals in the test article group were found dead just after dose administration for the sixth induction application. Tremors and salivation were noted in these animals prior to death. The three animals found dead were not included in the final evaluation, although the irritation scores for these animals are listed in Table 2. Due to the adverse effects in test article-treated animals by the end of the sixth induction application, no further induction applications were administered to avoid additional mortality. The initial dose mean irritation score for the test article group was zero and the group mean irritation score for all six induction applications was 0.29. The mean irritation score for the test article group at the challenge dose was 1.04. The large increase in irritation scores for the challenge dose compared to those for the induction phase is also indicative of a positive sensitization response.

A positive control group of ten animals using 2,4-Dinitrochlorobenzene, was induced in the same manner as the test article group. Subsequent challenge of the positive control group showed extreme sensitization with 10/10 animals showing a positive response. The initial dose mean irritation score for the positive control group was zero and the mean irritation score for all six induction applications was 0.23. The mean irritation score for the positive control group at the challenge dose was 1.33. The large increase in irritation noted at the challenge dose when compared to the induction phase is also indicative of a positive sensitization response in the positive control group.

Introduction

The object of this study was to determine the sensitization potential of hydroxyproplymethylcellulose gel containing 30% pyridostigmine bromide and 0.21% docusate sodium, in female albino guinea pigs. This study was conducted in accordance with the Food and Drug Administration's Good Laboratory Practice Regulation of 1978. The raw data generated by the Study Director and the final report are stored in the conducting laboratory's archives.

Method and Results

Twenty albino guinea pigs of the Hartley strain[a] were used to evaluate the sensitization potential of the hydroxypropylmethylcellulose gel containing 30% pyridostigmine bromide and 0.21% Docusate Sodium. All animals were held under quarantine for several days prior to testing and only animals which appeared to be in good health and suitable as test animals at the initiation of the study were used. The guinea pigs were housed in temperature and humidity controlled rooms and permitted a standard laboratory diet[b] and water ad libitum. The test method was a modification of Buehler[c].

The induction phase consisted of six topical applications of the test article[d] on the dorsal shoulder girdle of ten animals, which had been clipped free of hair prior to the application. The test article was secured with gauze and this in turn was firmly secured with elastic bandage material[f]. The patches were left in place for approximately a 24 hour contact period after which the patches and all residual test article were removed. Each application was then scored, on a scale of 0 to 4, for erythema and edema[g] (Table 1) at approximately 24 hours after each test article removal. Six topical applications of the test article at three applications per week (Monday, Wednesday and Friday) were applied to the dorsal shoulder girdle of the ten test guinea pigs. Fourteen days after

[a] Charles River Breeding Laboratories, Inc , Wilmington, MA
[b] Ralston Purina Guinea Pig Chow, Ralston Purina, St. Louis, MO
[c] Buehler, EV: Delayed Contact Hypersensitivity in the Guinea Pig. Arch. Dermat. 91:171 (1965)
[d] 0.1 ml dose for each application.
[e] Difco Labs, Inc., Detroit, MI
[f] Elastoplast , Beiersdorf, Inc., South Norwalk, CT
[g] Draize: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965).

the final application, the hair was removed from an area on the flank (posterior to the induction site) and the test article was applied in the same fashion as in the induction phase. This was left in place for approximately 24 hours. The challenge sites were evaluated one day and two days after removal of the patches, on a scale of 0 to 4 for erythema and edema[g].

The positive control (DNCB) group consisting of ten animals was treated in the same manner as the test article group, using 0.1 ml of 0.1% DNCB in propylene glycol for induction and 0.05% for the challenge. The results of the study are shown in Tables 2 and 3. The following grading system was used to arrive at a descriptive rating:

Sensitization Rate (%)	Classification
0 - 10	Weak
20 - 30	Mild
40 - 60	Moderate
70 - 80	Strong
90 - 100	Extreme

The protocol, principal personnel involved in the study, composition characteristics and Quality Assurance statement are contained in Appendices I-IV.

TABLE 1

SENSITIZATION TEST - ALBINO GUINEA PIGS

Scoring Criteria for Skin Reactions

Reaction	Description	Score
Erythema	Barely perceptible (Edges of area not defined)	1
	Pale red in color and area definable	2
	Definite red in color and area well defined.	3
	Beet or crimson red in color	4
Edema	Barely perceptible (Edges of area not defined)	1
	Area definable but not raised more 1 mm.	2
	Area well defined and raised approximately 1 mm.	3
	Area raised more than 1 mm.	4
	Maximum Primary Irritation Score	= 8

TABLE 2

Sensitization Study With Hydroxypropylmethylcellulose Gel Containing 30% Pyridostigmine Bromide and 0.21% Docusate Sodium in Albino Guinea Pigs
Results

Animal #	Dose # / Hour	1 24	2 24	3 24	4 24	5 24	6 24	6 Dose Average	Challenge Dose 24	Challenge Dose 48	
7G841	Erythema	0	0	0	1	1					
	Edema	0	0	0	0	0					
	Average	0	0	0	0.50	0.50	X				
7G847	Erythema	0	0	1	1	1	1		1	2	Group, Initial Dose Average = 0
	Edema	0	0	1	1	1	1		1	1	
	Average	0	0	1.00	1.00	1.00	1.00	0.67	1.25		
7G853	Erythema	0	0	0	0	2	2		2	2	
	Edema	0	0	0	0	1	1		1	1	
	Average	0	0	0	0	1.50	1.50	0.50	1.50		
7G859	Erythema	0	0	0	0	1	3		2	2	Group, 6 Dose Average = 0.29
	Edema	0	0	0	0	1	2		1	1	
	Average	0	0	0	0	1.00	2.50	0.58	1.50		
7G865	Erythema	0	0	0	0	0	1		2	2	
	Edema	0	0	0	0	0	1		1	1	
	Average	0	0	0	0	0	1.00	0.17	1.50		
7G842	Erythema	0	0	0	0	0	0		0	0	Group, Challenge Dose Average = 1.04
	Edema	0	0	0	0	0	0		0	0	
	Average	0	0	0	0	0	0	0	0.0		
7G848	Erythema	0	0	0	0	0	0		0	0	
	Edema	0	0	0	0	0	0		0	0	
	Average	0	0	0	0	0	0	0	0.0		
7G854	Erythema	0	0	0	0	0	1		2	2	
	Edema	0	0	0	0	0	0		1	1	
	Average	0	0	0	0	0	0.50	0.08	1.50		
7G860	Erythema	0	0	0	0	2					
	Edema	0	0	0	0	1					
	Average	0	0	0	0	1.50	X				
7G866	Erythema	0	0	0	1	2					
	Edema	0	0	0	1	1					
	Average	0	0	0	1.00	1.50	X				

X = Animal found dead (tremors and salivation noted prior to death)

5/7 animals positive - 71% Sensitization Rate = Grade IV or strong sensitizer.

Note: The values for animals found dead during the study are not used in the group average calculations.

TABLE 3

Sensitization Study With DNCB (Positive Control)
in Albino Guinea Pigs
Results

Animal #	Dose # / Hour	1 24	2 24	3 24	4 24	5 24	6 24	6 Dose Average	Challenge Dose 24	Challenge Dose 48
	Erythema	0	0	0	0	1	1		2	2
7G931	Edema	0	0	0	0	0	0		1	1
	Average	0	0	0	0	0.50	0.50	0.17	1.5	
	Erythema	0	0	0	1	1	1		2	2
7G937	Edema	0	0	0	0	0	0		1	1
	Average	0	0	0	0.50	0.50	0.50	0.25	1.5	
	Erythema	0	0	0	0	1	1		1	1
7G943	Edema	0	0	0	0	0	0		2	1
	Average	0	0	0	0	0.50	0.50	0.17	1.25	
	Erythema	0	0	0	1	1	3		2	2
7G949	Edema	0	0	0	0	1	2		1	1
	Average	0	0	0	0.50	1.00	2.50	0.67	1.5	
	Erythema	0	0	0	0	0	1		1	1
7G946	Edema	0	0	0	0	0	0		1	1
	Average	0	0	0	0	0	0.50	0.08	1.00	
	Erythema	0	0	0	0	0	0		1	1
7G932	Edema	0	0	0	0	0	0		1	1
	Average	0	0	0	0	0	0	0	1.00	
	Erythema	0	0	0	0	0	0		1	1
7G938	Edema	0	0	0	0	0	0		1	1
	Average	0	0	0	0	0	0	0	1.00	
	Erythema	0	0	0	1	1	3		2	2
7G944	Edema	0	0	0	0	0	2		2	2
	Average	0	0	0	0.50	0.50	2.50	0.58	2.00	
	Erythema	0	0	0	0	0	2		2	2
7G940	Edema	0	0	0	0	0	1		1	1
	Average	0	0	0	0	0	1.50	0.25	1.5	
	Erythema	0	0	0	0	0	1		1	1
7G934	Edema	0	0	0	0	0	0		1	1
	Average	0	0	0	0	0	0.50	0.08	1.00	

Group, Initial Dose Average = 0

Group, 6 Dose Average = 0.23

Group, Challenge Dose Average = 1.33

10/10 animals positive - 100% Sensitization Rate = Grade V or extreme sensitizer.

TEST: Modified Buehler[a] Sensitization Test

SPONSOR: 3M RIKER Division

CONDUCTED BY: **Pathology and Toxicology Department, Riker Laboratories, Inc., St. Paul, Minnesota**

TEST ARTICLE: Hydroxypropylmethylcellulose gel containing 3% Pyridostigmine Bromide and 0.2% Docusate Sodium, Lot FN4589

POSITIVE CONTROL ARTICLE: 2,4 - Dinitrochlorobenzene

PROPOSED STARTING/COMPLETION DATE OF TEST: 3/87 - 7/87

TEST SYSTEM: Hartley Strain, Guinea Pig (of either sex)

AGE: 6-10 weeks

SOURCE: Charles River Breeding Laboratories, Wilmington, Massachusetts

---

OBJECTIVE: To determine the sensitization potential of the test article. Guinea pigs will be used as the test system due to their historical use and ease of handling.

METHOD: The method will be a modification of Buehler[a]. The animals will be housed in standard cages in temperature and humidity controlled rooms with food[b] and water offered **ad libitum**. The animals' numbers will be placed on cards affixed to the outside of their cages. The test will be conducted in two phases; an induction phase and a challenge phase. The induction phase will consist of nine topical applications of the test article[c] at three applications per week (Monday, Wednesday and Friday) in the dorsal shoulder girdle, which will be clipped free of hair prior to the application procedure. A group of ten animals will be administered the test article and an additional group of ten animals will be administered the positive control[d]. The test article will be applied and firmly secured. The test article will be left in place for approximately 24 hours, after which all residual test material will be removed. The subsequent applications will be done in the same manner as the initial application. The positive control group will be dosed in the same manner as the test article group. Each animal will be evaluated[e] for signs of skin irritation approximately 24 hours after removal of the test article for each of the nine exposures. Two weeks after the induction phase is complete, the hair will be removed from an area on the flank (posterior to the induction site). The test article will be applied to the site,

PROTOCOL (continued)

METHOD: secured and left in place for approximately 24 hours. The challenge sites will be evaluated approximately 24 and 48 hours after removal of all test articles. All skin reactions will be scored on the basis of 0 to 4[e].

[a] Buehler, EV; Delayed Contact Hypersensitivity in the Guinea Pig, Arch. Dermat. 91:171 (1965)
[b] Purina Guinea Pig Chow, Ralston Purina Co., St. Louis, Missouri
[c] The test article dose will be 0.1 ml
[d] 0.1 ml of 2,4-dinitrochlorobenzene, Sigma Chemical Co., St. Louis, Missouri
[e] Draize: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965)

Marie McKay 2/27/87 Gene L Harris 3/2/87
Sponsor Date Study Director Date

Riker Experiment No. 0387MG0055

Appendix I (concluded)
Deviations and/or Amendments to Protocol

1. The induction phase will be limited to 6 doses. Reason for change: to avoid mortality in the study animals due to test material.

Gene L. Harris. 3/17/87
Study Director Date

2. Pyridostigmine bromide may also be named by "S-26741" in the raw data of this study. Reason for change: S-26741 in the Riker # given to pyridostigmine bromide.

Gene L. Harris 4/14/87
Study Director Date

3.

Study Director Date

4.

Study Director Date

APPENDIX II

Principal Participating Personnel Involved in the Study

Name	Function
G. L. Harris, B.S.	Advanced Toxicologist Study Director
G. E. Hart	Master Laboratory Technician Acute Toxicology
L. O. Wiseth	Sr. Laboratory Technician Acute Toxicology
J. A. Eads	Jr. Laboratory Technician Acute Toxicology
J. L. Allen, Ph.D.	Research Toxicologist Acute Toxicology
G. C. Pecore	Supervisor Animal Laboratory

APPENDIX III

Test and/or Control Article Characterization

for

Hydroxypropylmethylcellulose gel containing 30% pyridostigmine Bromi
and 0.2% Docusate Sodium, (FN 4589)

1. The identity strength, uniformity, composition, purity or other pertinent characterizations of the test and/or substances have been determined and documented as of RFA 14201 – 2/27/87.

2. The method of synthesis or origin of the test and control substances, including their amount and the method of bioassay (if applicable) is documented.

   ☐ Yes ☐ No Anita Tilton 2/27/87

3. The stability of the test and/or control substances* ~~have been determined or~~ will be determined ~~at~~ AT of the end of the study.

   Anita Tilton 2/27/87

The above information and documentation are located in the sponsor's records.

~~Sponsor or~~ Sponsor Representative	Date
Anita Tilton	2/27/87

Original Characterization can be found in Exp# 0387MG0054

* = Form change

Form 19793-A-PWO

APPENDIX IV

QUALITY ASSURANCE STATEMENT

Acute Toxicology Laboratory Studies

Study No.: 0387MG0055

This short term study was audited by Compliance Audit and the final report examined against the raw data on April 21, 1987. The results of the audit were reported to the study director and to management on April 21, 1987.

In addition to the data audit, different significant phases for studies underway in the Acute Toxicology Laboratory are inspected on a recurring cycle, and the facilities are examined by Compliance Audit on a three month schedule.

D. M. Warboard
Compliance Audit

4-21-87
Date

Pathology and Toxicology
Riker Laboratories, Inc.

Building 270-3S-05, 3M Center
St. Paul, Minnesota 55144-1000

COMPANY CONFIDENTIAL

3M

Sensitizaton Study with Hydroxypropylmethylcellulose Gel Containing 30% Pyridostigmine Bromide and 0.198% Sodium Lauryl Sulfate in Albino Guinea Pigs

Riker Experiment No: 0387MG0057

Conducted At: Pathology and Toxicology
Riker Laboratories, Inc.

Dates Conducted: March 4, 1987 to April 2, 1987

Conducted By: Glenn L. Harris 4/22/87
G. L. Harris, B.S. Date
Advanced Toxicologist
Study Director and Acute Toxicity
Study Coordinator

Reviewed By: J Allen 4-22-87
J. L. Allen, Ph.D. Date
Diplomate, A.B.T.
Research Toxicologist

dc: R.T. Catherall
M.W. Downing
N.M. Marecki
M.J. Westfall (2)
Tech. Doc. Center
Path/Tox File

Summary

A skin sensitization study was conducted from March 4, 1987 to April 2, 1987 at Riker Laboratories, Inc., St. Paul, Minnesota. The results indicate that hydroxypropylmethylcellulose gel containing 30% pyridostigmine bromide and 0.198% sodium lauryl sulfate is a potentially strong sensitizer with positive responses noted in 6/8 animals. An individual animal was considered positive in this test if irritation was noted in the animal at the challenge dose evaluation. The initial induction dose application score for each animal in this study was zero.

All animals in all groups in this study received two, 0.1 ml intradermal injections of Freund's complete adjuvant just prior to the initial induction dose.

Ten female albino guinea pigs in the test article group received six topical induction applications of the potential antigen and subsequently challenged topically 14 days post induction. Two animals in the test article group were found dead after dose administration of the sixth induction application. Tremors and salivation were noted in these animals prior to death. The two animals found dead are not included in the final evaluation, although the irritation scores for this animal are listed in Table 2. Due to the adverse effects noted in the animals by the end of the sixth induction application, no further induction applications were administered to avoid additional mortality of the study animals. The initial dose mean irritation score for the test article group was zero and the mean irritation score for all six induction applications was 0.41. The mean irritation score for the challenge dose was 0.88. The large increase in irritation scores at the challenge dose compared to the induction phase is also indicative of a positive sensitization response.

A sham control group of ten animals received the initial application of two 0.1 ml intradermal injections of Freund's complete adjuvant without being induced with the six induction applications of the test material. The sham control group received the same dose, exposure and observations as the test article group during the challenge phase of the study. Minimal erythema was noted in 1/10 animals in the sham control group at the challenge dose evaluation. The group mean score for the challenge dose evaluation was 0.05. The results indicate the administration of adjuvant did not significantly influence the degree of irritation noted in the test article group at the challenge dose evaluation.

A positive control group of ten animals using 2,4-Dinitrochlorobenezene, was induced in the same manner as the test article group. Subsequent challenge of the positive control group showed extreme sensitization with 10/10 animals showing a positive response. The group initial dose mean irritation score for the positive control group was zero and the group mean irritation score for all six induction applications was 0.38. The mean irritation score for the positive control group at the challenge dose was 1.70. The large increase in irritation noted at the challenge dose when compared to the induction phase is also indicative of a positive sensitization response in the positive control group.

Introduction

The object of this study was to determine the sensitization potential of hydroxypropylmethylcellulose gel containing 30% pyridostigmine bromide and 0.198% sodium lauryl sulfate in female albino guinea pigs. This study was conducted in accordance with the Food and Drug Administration's Good Laboratory Practice Regulation of 1978. The raw data generated by the Study Director and the final report are stored in the conducting laboratory's archives.

Method and Results

Thirty albino guinea pigs of the Hartley strain[a] were used to evaluate the sensitization potential of the hydroxypropylmethylcellulose gel containing 30% pyridostigmine bromide and 0.198% sodium lauryl sulfate. All animals were held under quarantine for several days prior to testing and only animals which appeared to be in good health and suitable as test animals at the initiation of the study were used. The guinea pigs were housed in temperature and humidity controlled rooms and permitted a standard laboratory diet[b] and water ad libitum. The test method was a modification of Buehler[c].

The induction phase consisted of six topical applications of the test article[d] on the dorsal shoulder girdle of ten animals, which had been clipped free of hair prior to the application. The initial topical application also included two 0.1 ml intradermal injections of Freund's Adjuvant[e] (1:1 commercial adjuvant with water) close to the area where the test article was immediately applied. The patches were secured with gauze and this in turn was firmly secured with elastic bandage material[f]. The patches were left in place for an approximately 24 hour contact period after which the patches and all residual test article were removed. Each application was then scored on a scale of 0 to 4 for erythema and edema[g] (Table 1) at approximately 24 hours after each test article removel. Six topical applications of the test article at three applications per week (Monday, Wednesday and Friday) were applied to the dorsal shoulder girdle of the ten test guinea pigs. Fourteen days after the final application,

[a] Charles River Breeding Laboratories, Inc., Wilmington, MA
[b] Ralston Purina Guinea Pig Chow, Ralston Purina, St. Louis, MO
[c] Buehler, EV: Delayed Contact Hypersensitivity in the Guinea Pig. Arch. Dermat. 91:171 (1965)
[d] Approximately 0.1 ml dose for each application
[e] Difco Labs, Inc., Detroit, MI
[f] Elastoplast , Beiersdorf, Inc., South Norwalk, CT
[g] Draize: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965).

the hair was removed from an area on the flank (posterior to the induction site) and the test article applied in the same fashion as for the induction phase. This was left in place for approximately 24 hours. The challenge sites were evaluated one day and two days after removal of the patches, on a scale of 0 to 4 for erythema and edema.

The positive control (DNCB)[h] group consisting of ten animals was treated in the same manner as the test article group, using 0.1 ml of 0.1% DNCB in propylene glycol for induction and 0.05% for the challenge. A sham control group of ten animals received the initial application of two 0.1 ml intradermal injections of Freund's complete adjuvant without being induced with the six induction applications of the test material. The sham control group received the same dose, exposure and observations as the test article group during the challenge dose phase of the study. The results of the study are shown in Tables 2-4. The following grading system was used to arrive at a descriptive rating:

Sensitization Rate (%)	Classification
0 - 10	Weak
20 - 30	Mild
40 - 60	Moderate
70 - 80	Strong
90 - 100	Extreme

The protocol, principal personnel involved in the study, composition characteristics and Quality Assurance statement are contained in Appendices I-IV.

[h] 2,4-Dinitrochlorobenzene, Kodak, Lot A11G

TABLE 1

SENSITIZATION TEST - ALBINO GUINEA PIGS

Scoring Criteria for Skin Reactions

Reaction	Description	Score
Erythema	Barely perceptible (Edges of area not defined)	1
	Pale red in color and area definable	2
	Definite red in color and area well defined.	3
	Beet or crimson red in color	4
Edema	Barely perceptible (Edges of area not defined)	1
	Area definable but not raised more 1 mm.	2
	Area well defined and raised approximately 1 mm.	3
	Area raised more than 1 mm.	4
	Maximum Primary Irritation Score	= 8

TABLE 2

Guinea Pig Skin Sensitization Study With Hydroxypropylmethylcellulose Gel Containing 30% Pyridostigmine Bromide and 0.198% Sodium Lauryl Sulfate (Test Article Group)
Results

Animal #	Dose # / Hour	1 24	2 24	3 24	4 24	5 24	6 24	6 Dose Average	Challenge Dose 24	Challenge Dose 48	
	Erythema	0	0	0	1	3					
7G873	Edema	0	0	0	0	1					
	Average	0	0	0	0.50	2.00	X				
	Erythema	0	0	0	0	0	1		2	2	Group, Initial Dose
7G879	Edema	0	0	0	0	0	0		1	1	Average = 0
	Average	0	0	0	0.00	0.00	0.50	0.08	1.50		
	Erythema	0	0	0	0	0	1		1	1	
7G885	Edema	0	0	0	0	0	0		0	0	
	Average	0	0	0	0.00	0.00	0.50	0.08	0.50		
	Erythema	0	0	1	1	1	2		0	0	Group, 6 Dose
7G891	Edema	0	0	1	1	1	2		0	0	Average = 0.41
	Average	0	0	1.00	1.00	1.00	2.00	0.83	0.00		
	Erythema	0	0	1	1	2					
7G897	Edema	0	0	0	0	1					
	Average	0	0	0.50	0.50	1.50	X				
	Erythema	0	0	0	0	1	1		2	2	Group, Challenge
7G874	Edema	0	0	0	0	0	1		1	1	Dose Average = 0.88
	Average	0	0	0	0.00	0.50	1.00	0.25	1.50		
	Erythema	0	0	1	1	1	1		1	1	
7G880	Edema	0	0	1	1	1	1		0	0	
	Average	0	0	1.00	1.00	1.00	1.00	0.67	0.50		
	Erythema	0	0	1	1	1	1		0	0	
7G886	Edema	0	0	1	1	1	1		0	0	
	Average	0	0	1.00	1.00	1.00	1.00	0.67	0.00		
	Erythema	0	0	1	1	1	1		2	2	
7G892	Edema	0	0	1	1	1	1		2	2	
	Average	0	0	1.00	1.00	1.00	1.00	0.67	2.00		
	Erythema	0	0	0	0	0	0		1	1	
7G898	Edema	0	0	0	0	0	0		1	1	
	Average	0	0	0	0.00	0.00	0.00	0.00	1.00		

X = Animal found dead (salivation and tremors noted prior to death)

6/8 animals positive - 75% Sensitization Rate = Grade IV or strong sensitizer.

Note: The values for animals found dead during the study are not used in the group average calculations.

TABLE 3

Guinea Pig Skin Sensitization Study With Hydroxypropylmethylcellulose Gel Containing 30% Pyridostigmine Bromide and 0.198% Sodium Lauryl Sulfate (Sham Control Group) Results

Animal #	Hour	Challenge Dose 24	48
7G903	Erythema	0	0
	Edema	0	0
	Average	0	
7G909	Erythema	1	1
	Edema	0	0
	Average	0.5	
7G915	Erythema	0	0
	Edema	0	0
	Average	0	
7G921	Erythema	0	0
	Edema	0	0
	Average	0	
7G927	Erythema	0	0
	Edema	0	0
	Average	0	
7G904	Erythema	0	0
	Edema	0	0
	Average	0	
7G910	Erythema	0	0
	Edema	0	0
	Average	0	
7G916	Erythema	0	0
	Edema	0	0
	Average	0	
7G922	Erythema	0	0
	Edema	0	0
	Average	0	
7G928	Erythema	0	0
	Edema	0	0
	Average	0	

Group Challenge Dose Average = 0

TABLE 4

Guinea Pig Skin Sensitization Study With DNCB (Positive Control Group)
Results

Animal #	Dose # / Hour	1 24	2 24	3 24	4 24	5 24	6 24	6 Dose Average	Challenge Dose 24	Challenge Dose 48
	Erythema	0	0	0	1	1	3		2	2
7G929	Edema	0	0	0	0	0	2		1	1
	Average	0	0	0	0.50	0.50	2.50	0.58	1.5	
	Erythema	0	0	0	0	1	1		2	2
7G935	Edema	0	0	0	0	0	0		2	2
	Average	0	0	0	0.00	0.50	0.50	0.17	2.0	
	Erythema	0	0	0	2	2	3		3	3
7G941	Edema	0	0	0	1	1	2		2	2
	Average	0	0	0	1.50	1.50	2.50	0.92	2.50	
	Erythema	0	0	0	0	1	1		2	3
7G947	Edema	0	0	0	0	0	0		2	2
	Average	0	0	0	0.00	0.50	0.50	0.17	2.25	
	Erythema	0	0	0	0	1	1		2	2
7G950	Edema	0	0	0	0	0	0		1	2
	Average	0	0	0	0.00	0.50	0.50	0.17	1.75	
	Erythema	0	0	0	1	1	2		2	2
7G930	Edema	0	0	0	1	1	1		1	1
	Average	0	0	0	1.00	1.00	1.50	0.58	1.50	
	Erythema	0	0	0	0	0	0		1	1
7G936	Edema	0	0	0	0	0	0		1	1
	Average	0	0	0	0.00	0.00	0.00	0.00	1.00	
	Erythema	0	0	0	0	1	3		2	2
7G942	Edema	0	0	0	0	0	2		1	1
	Average	0	0	0	0.00	0.50	2.50	0.50	1.50	
	Erythema	0	0	0	1	2	2		2	2
7G948	Edema	0	0	0	0	0	0		1	1
	Average	0	0	0	0.50	1.00	1.00	0.42	1.50	
	Erythema	0	0	0	0	1	2		2	2
7G945	Edema	0	0	0	0	0	1		1	1
	Average	0	0	0	0.00	0.50	1.50	0.33	1.50	

Group, Initial Dose Average = 0

Group, 6 Dose Average = 0.38

Group, Challenge Dose Average = 1.70

10/10 animals positive - 100% Sensitization Rate = Grade V or extreme sensitizer.

TEST: Modified Buehler[a] Sensitization Test

SPONSOR: 3M RIKER Division

CONDUCTED BY: **Pathology and Toxicology Department, Riker Laboratories, Inc., St. Paul, Minnesota**

TEST ARTICLE: Hydroxypropylmethylcellulose gel containing 30% pyridostigmine bromide and 0.198% sodium lauryl sulfate, Lot FN4590

POSITIVE CONTROL ARTICLE: 2,4 - Dinitrochlorobenzene

PROPOSED STARTING/COMPLETION DATE OF TEST: 3/87 - 7/87

TEST SYSTEM: Hartley Strain, Guinea Pig (of either sex)

AGE: 6-10 weeks

SOURCE: Charles River Breeding Laboratories, Wilmington, Massachusetts

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OBJECTIVE: To determine the sensitization potential of the test article. Guinea pigs will be used as the test system due to their historical use and ease of handling.

METHOD: The method will be a modification of Buehler[a]. The animals will be housed in standard cages in temperature and humidity controlled rooms with food[b] and water offered **ad libitum**. The animals' numbers will be placed on cards affixed to the outside of their cages. The test will be conducted in two phases; an induction phase and a challenge phase. The induction phase will consist of nine topical applications of the test article[c] at three applications per week (Monday, Wednesday and Friday) in the dorsal shoulder girdle, which will be clipped free of hair prior to the application procedure. The initial procedure will consist of two injections of 0.1 ml (per injection - one/side) of Freund's Adjuvant[d] (1:1 commercial adjuvant with water) in each of the ten animals that will be administered the test article and in each of the ten animals that will be administered the positive control[e]. The test article will be placed near the injection sites and firmly secured. The test article will be left in place for approximately 24 hours, after which all residual test material will be removed. The subsequent applications will be done in the same manner as the initial application excluding the injection of adjuvant. The positive control group will be dosed in the same manner as the test article group. Each animal will be evaluated[f] for signs of skin irritation approximately 24 hours after removal of the test article for each of the nine exposures. Two weeks after the induction phase is complete, the hair will be removed from an area on the flank (posterior to the induction site). The test article will be applied to the site, secured and left in place for approximately 24 hours. The challenge sites will be evaluated approximately 24 and 48 hours after removal of all test articles. All skin reactions will be scored on the basis of 0 to 4[f]. An additional sham control group of ten animals will

PROTOCOL (continued)

METHOD: receive the initial application of two injections of 0.1 ml of Freund's adjuvant without being induced with the nine induction applications of the test material. The sham control group will receive the same dose, exposure and observations as the test article group during the challenge dose phase of the study. The sham control group and the test article group will be utilized to compare any differences between the two groups in severity of skin irritation present at the challenge dose site. The comparison should allow the study director to separate irritation caused by the test material that may be present only because the animals received adjuvant alone from irritation that is indicative of sensitization due to the nine induction applications. All raw data generated by the study director and the final report will be stored in Riker Laboratories' Archives, St. Paul, Minnesota.

a- Buehler, EV; Delayed Contact Hypersensitivity in the Guinea Pig, Arch. Dermat. 91:171 (1965)
b- Purina Guinea Pig Chow, Ralston Purina Co., St. Louis, Missouri
c- The test article dose will be 0.1 ml
d- Difco Laboratories, Inc., Detroit, Michigan
e- 0.1 ml of 2,4-dinitrochlorobenzene, Sigma Chemical Co., St. Louis, Missouri
f- Draize: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965)

Mari Ellen Hall 2/27/87
Sponsor Date

Glenn L. Harris 3/1/87
Study Director Date

Riker Experiment No. 0387MG0057

Appendix I (concluded)
Deviations and/or Amendments to Protocol

1. The induction phase will be limited to 6 doses. Reason for change: to avoid mortality in the study animals due to test material.

Gene L. Harris — 3/17/87
Study Director — Date

2. Pyridostigmine bromide may also be named "S-26741" in this study. Reason for change: S-26741 is the Riker name given to pyridostigmine bromide.

Gene L. Harris — 4/16/87
Study Director — Date

3.

Study Director — Date

4.

Study Director — Date

APPENDIX II

Principal Participating Personnel Involved in the Study

Name	Function
G. L. Harris, B.S.	Advanced Toxicologist Study Director
G. E. Hart	Master Laboratory Technician Acute Toxicology
L. O. Wiseth	Sr. Laboratory Technician Acute Toxicology
J. A. Eads	Jr. Laboratory Technician Acute Toxicology
J. L. Allen, Ph.D.	Research Toxicologist Acute Toxicology
G. C. Pecore	Supervisor Animal Laboratory

APPENDIX III

Test and/or Control Article Characterization

for

Hydroxypropyl methylcellulose gel containing 30% pyridostigmine Bromide and 0.198% Sodium Lauryl Sulfate, (FN 4590)

1. The identity strength, uniformity, composition, purity or other pertinent characterizations of the test and/or substances have been determined and documented as of RFA 14202-2/27/87.

2. The method of synthesis or origin of the test and control substances, including their amount and the method of bioassay (if applicable) is documented.

☐ Yes ☐ No Amit w Tilm 2/27/87

3. The stability of the test and/or control substances ~~have been determined~~ * ~~or~~ will be determined ~~as of~~ The end of the Study. AT Amit w Tilm 2/27/87

The above information and documentation are located in the sponsor's records.

~~Sponsor or~~ Sponsor Representative	Date
Amit w Tilm	2/27/87

* = Form change

APPENDIX IV

QUALITY ASSURANCE STATEMENT

Acute Toxicology Laboratory Studies

Study No.: 0387MG0057

This short term study was audited by Compliance Audit and the final report examined against the raw data on April 21, 1987. The results of the audit were reported to the study director and to management on April 21, 1987.

In addition to the data audit, different significant phases for studies underway in the Acute Toxicology Laboratory are inspected on a recurring cycle, and the facilities are examined by Compliance Audit on a three month schedule.

D. M. Markow

Compliance Audit

4-21-87

Date

Pathology and Toxicology
Riker Laboratories, Inc.

Building 270-3S-05, 3M Center
St. Paul, Minnesota 55144-1000

COMPANY CONFIDENTIAL

3M

Sensitizaton Study with Hydroxypropylmethylcellulose Gel Containing 30% Pyridostigmine Bromide and 0.198% Sodium Lauryl Sulfate in Albino Guinea Pigs

Riker Experiment No: 0387MG0058

Conducted At: Pathology and Toxicology
Riker Laboratories, Inc.

Dates Conducted: March 4, 1987 to April 2, 1987

Conducted By: Glen L. Harris 4/22/87
G. L. Harris, B.S. Date
Advanced Toxicologist
Study Director and Acute Toxicity
Study Coordinator

Reviewed By: J. Allen 4-22-87
J. L. Allen, Ph.D. Date
Diplomate, A.B.T.
Research Toxicologist

dc: R.T. Catherall
M.W. Downing
N.M. Marecki
M.J. Westfall (2)
Tech. Doc. Center
Path/Tox Files

Summary

A skin sensitization study was conducted from March 4, 1987 to April 2, 1987, at Riker Laboratories, Inc., St. Paul, Minnesota. The results indicate that Hydroxypropylmethyl cellulose gel containing 30% pyridostigmine and 0.198% sodium lauryl sulfate is a potentially extreme sensitizer with positive responses noted in 8/9 animals. An individual animal was considered positive in this test if irritation was noted in the animal at the challenge dose evaluation. The initial induction dose application score for each animal in this study was zero.

Ten female albino guinea pigs in the test article group received six topical induction applications of the potential antigen and subsequently challenged topically 14 days post induction. One animal in the test article group was found dead just after dose administration for the sixth induction application. Tremors and salivation were noted in the animal prior to death. The animal found dead was not included in the final evaluation, although the irritation scores for the animal are listed in Table 2. Due to the adverse effects in test article-treated animals by the end of the sixth induction application, no further induction applications were administered to avoid additional mortality. The initial dose mean irritation score for the test article group was zero and the mean irritation score for all six induction applications was 0.38. The mean irritation score for the test article group at the challenge dose was 1.17. The large increase in irritation scores for the challenge dose compared to those for the induction phase is also indicative of a positive sensitization response.

A positive control group of ten animals using 2,4-Dinitrochlorobenzene, was induced in the same manner as the test article group. Subsequent challenge of the positive control group showed extreme sensitization with 10/10 animals showing a positive response. The initial dose mean irritation score for the positive control group was zero and the mean irritation score for all six induction applications was 0.23. The mean irritation score for the positive control group at the challenge dose was 1.33. The large increase in irritation noted at the challenge dose when compared to the induction phase is also indicative of a positive sensitization response in the positive control group.

Introduction

The object of this study was to determine the sensitization potential of hydroxypropylmethylcellulose gel containing 30% pyridostigmine bromide and 0.198% sodium lauryl sulfate, in female albino guinea pigs. This study was conducted in accordance with the Food and Drug Administration's Good Laboratory Practice Regulation of 1978. The raw data generated by the Study Director and the final report are stored in the conducting laboratory's archives.

Method and Results

Twenty albino guinea pigs of the Hartley strain[a] were used to evaluate the sensitization potential of the hydroxypropylmethylcellulose gel containing 30% pyridostigmine bromide and 0.198% sodium lauryl sulfate. All animals were held under quarantine for several days prior to testing and only animals which appeared to be in good health and suitable as test animals at the initiation of the study were used. The guinea pigs were housed in temperature and humidity controlled rooms and permitted a standard laboratory diet[b] and water ad libitum. The test method was a modification of Buehler[c].

The induction phase consisted of six topical applications of the test article[d] on the dorsal shoulder girdle of ten animals, which had been clipped free of hair prior to the application. The test article was secured with gauze and this in turn was firmly secured with elastic bandage material[f]. The patches were left in place for approximately a 24 hour contact period after which the patches and all residual test article were removed. Each application was then scored on a scale of 0 to 4 for erythema and edema[g] (Table 1) at approximately 24 hours after each test article removal. Six topical applications of the test article at three applications per week (Monday, Wednesday and Friday) were applied to the dorsal shoulder girdle of the ten guinea pigs. Fourteen days after the

[a] Charles River Breeding Laboratories, Inc., Wilmington, MA
[b] Ralston Purina Guinea Pig Chow, Ralston Purina, St. Louis, MO
[c] Buehler, EV: Delayed Contact Hypersensitivity in the Guinea Pig. Arch. Dermat. 91:171 (1965)
[d] 0.1 ml dose for each application
[e] Difco Labs, Inc., Detroit, MI
[f] Elastoplast , Beiersdorf, Inc., South Norwalk, CT
[g] Draize: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965).

final induction application, the hair was removed from an area on the flank (posterior to the induction site) and the test article was applied in the same fashion as in the induction phase. This was left in place for approximately 24 hours. The challenge sites were evaluated one day and two days after removal of the patches, on a scale of 0 to 4 for erythema and edema.

The positive control (DNCB) group consisting of ten animals was treated in the same manner as the test article group, using 0.1 ml of 0.1% DNCB in propylene glycol for induction and 0.05% for the challenge. The results of the study are shown in Tables 2 and 3. The following grading system was used to arrive at a descriptive rating:

Sensitization Rate (%)	Classification
0 - 10	Weak
20 - 30	Mild
40 - 60	Moderate
70 - 80	Strong
90 - 100	Extreme

The protocol, principal personnel involved in the study, composition characteristics and Quality Assurance statement are contained in Appendices I-IV.

TABLE 1

SENSITIZATION TEST - ALBINO GUINEA PIGS

Scoring Criteria for Skin Reactions

Reaction	Description	Score
Erythema	Barely perceptible (Edges of area not defined)	1
	Pale red in color and area definable	2
	Definite red in color and area well defined.	3
	Beet or crimson red in color	4
Edema	Barely perceptible (Edges of area not defined)	1
	Area definable but not raised more 1 mm.	2
	Area well defined and raised approximately 1 mm.	3
	Area raised more than 1 mm.	4
	Maximum Primary Irritation Score	= 8

TABLE 2

Sensitization Study With Hydroxypropylmethylcellulose Gel Containing 30% Pyridostigmine Bromide and 0.198% Sodium Lauryl Sulfate
Results

Animal #	Dose # / Hour	1 24	2 24	3 24	4 24	5 24	6 24	6 Dose Average	Challenge Dose 24	Challenge Dose 48
	Erythema	0	0	0	0	0	1		1	1
7G843	Edema	0	0	0	0	0	1		1	1
	Average	0	0	0	0	0	1.00	0.17	1.00	
	Erythema	0	0	0	0	2	3		3	3
7G849	Edema	0	0	0	0	1	2		2	2
	Average	0	0	0	0	1.50	2.50	0.67	2.50	
	Erythema	0	0	0	0	0	3		1	2
7G855	Edema	0	0	0	0	0	2		1	1
	Average	0	0	0	0	0	2.50	0.42	1.25	
	Erythema	0	0	0	0	0	3		1	1
7G861	Edema	0	0	0	0	0	2		0	0
	Average	0	0	0	0	0	2.5	0.42	0.50	
	Erythema	0	0	0	0	1	2		1	1
7G867	Edema	0	0	0	0	0	1		0	0
	Average	0	0	0	0	0.50	1.50	0.33	0.50	
	Erythema	0	0	0	1	1	2		2	2
7G844	Edema	0	0	0	1	1	2		2	2
	Average	0	0	0	1.00	1.00	2.00	0.67	2.00	
	Erythema	0	0	0	0	0	2		2	2
7G850	Edema	0	0	0	0	0	2		2	2
	Average	0	0	0	0	0	2.00	0.33	2.00	
	Erythema	0	0	0	0	0	0		0	0
7G856	Edema	0	0	0	0	0	0		0	0
	Average	0	0	0	0	0	0	0.00	0.00	
	Erythema	0	0	0	0	1	2		1	1
7G862	Edema	0	0	0	0	0	2		0	1
	Average	0	0	0	0	0.50	2.00	0.42	0.75	
	Erythema	0	0	0	1	2				
7G868	Edema	0	0	0	1	1				
	Average	0	0	0	1.00	1.50	X		0.0	

Group, Initial Dose Average = 0

Group, 6 Dose Average = 0.38

Group, Challenge Dose Average = 1.17

X = Animal found dead (tremors and salivation noted prior to death)

8/9 animals positive - 88% Sensitization Rate = Grade V or extreme sensitizer.

Note: The values for animals found dead during the study are not included in the group average calculations.

TABLE 3

Sensitization Study With DNCB (Positive Control)
in Albino Guinea Pigs
Results

Animal #	Dose # / Hour	1 24	2 24	3 24	4 24	5 24	6 24	6 Dose Average	Challenge Dose 24	Challenge Dose 48
	Erythema	0	0	0	0	1	1		2	2
7G931	Edema	0	0	0	0	0	0		1	1
	Average	0	0	0	0	0.50	0.50	0.17	1.5	
	Erythema	0	0	0	1	1	1		2	2
7G937	Edema	0	0	0	0	0	0		1	1
	Average	0	0	0	0.50	0.50	0.50	0.25	1.5	
	Erythema	0	0	0	0	1	1		1	1
7G943	Edema	0	0	0	0	0	0		2	1
	Average	0	0	0	0	0.50	0.50	0.17	1.25	
	Erythema	0	0	0	1	1	3		2	2
7G949	Edema	0	0	0	0	1	2		1	1
	Average	0	0	0	0.50	1.00	2.50	0.67	1.5	
	Erythema	0	0	0	0	0	1		1	1
7G946	Edema	0	0	0	0	0	0		1	1
	Average	0	0	0	0	0	0.50	0.08	1.00	
	Erythema	0	0	0	0	0	0		1	1
7G932	Edema	0	0	0	0	0	0		1	1
	Average	0	0	0	0	0	0	0	1.00	
	Erythema	0	0	0	0	0	0		1	1
7G938	Edema	0	0	0	0	0	0		1	1
	Average	0	0	0	0	0	0	0	1.00	
	Erythema	0	0	0	1	1	3		2	2
7G944	Edema	0	0	0	0	0	2		2	2
	Average	0	0	0	0.50	0.50	2.50	0.58	2.00	
	Erythema	0	0	0	0	0	2		2	2
7G940	Edema	0	0	0	0	0	1		1	1
	Average	0	0	0	0	0	1.50	0.25	1.5	
	Erythema	0	0	0	0	0	1		1	1
7G934	Edema	0	0	0	0	0	0		1	1
	Average	0	0	0	0	0	0.50	0.08	1.00	

Group, Initial Dose Average = 0

Group, 6 Dose Average = 0.23

Group, Challenge Dose Average = 1.33

10/10 animals positive - 100% Sensitization Rate = Grade V or extreme sensitizer.

RIKER Experiment No.: 0287MG0058

APPENDIX I

PROTOCOL

8.

931

TEST: Modified Buehler[a] Sensitization Test

SPONSOR: 3M RIKER Division

CONDUCTED BY: **Pathology and Toxicology Department, Riker Laboratories, Inc., St. Paul, Minnesota**

TEST ARTICLE: Hydroxy propyl methyl cellulose gel containing 30% Pyridostigmine Bromide and 0.198% Sodium Lauryl Sulfate, Lot FN4590

POSITIVE CONTROL ARTICLE: 2,4 - Dinitrochlorobenzene

PROPOSED STARTING/COMPLETION DATE OF TEST: 3/87 - 7/87

TEST SYSTEM: Hartley Strain, Guinea Pig (of either sex)

AGE: 6-10 weeks

SOURCE: Charles River Breeding Laboratories, Wilmington, Massachusetts

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OBJECTIVE: To determine the sensitization potential of the test article. Guinea pigs will be used as the test system due to their historical use and ease of handling.

METHOD: The method will be a modification of Buehler[a]. The animals will be housed in standard cages[b] in temperature and humidity controlled rooms with food and water offered **ad libitum**. The animals' numbers will be placed on cards affixed to the outside of their cages. The test will be conducted in two phases; an induction phase and a challenge phase. The induction phase will consist of nine topical applications of the test article[c] at three applications per week (Monday, Wednesday and Friday) in the dorsal shoulder girdle, which will be clipped free of hair prior to the application procedure. A group of ten animals will be administered the test article and an additional group of ten animals will be administered the positive control[d]. The test article will be applied and firmly secured. The test article will be left in place for approximately 24 hours, after which all residual test material will be removed. The subsequent applications will be done in the same manner as the initial application. The positive control group will be dosed in the same manner as the test article group. Each animal will be evaluated[e] for signs of skin irritation approximately 24 hours after removal of the test article for each of the nine exposures. Two weeks after the induction phase is complete, the hair will be removed from an area on the flank (posterior to the induction site). The test article will be applied to the site,

PROTOCOL (continued)

METHOD: secured and left in place for approximately 24 hours. The challenge sites will be evaluated approximately 24 and 48 hours after removal of all test articles. All skin reactions will be scored on the basis of 0 to 4[e].

[a] Buehler, EV; Delayed Contact Hypersensitivity in the Guinea Pig, Arch. Dermat. 91:171 (1965)
[b] Purina Guinea Pig Chow, Ralston Purina Co., St. Louis, Missouri
[c] The test article dose will be 0.1 ml
[d] 0.1 ml of 2,4-dinitrochlorobenzene, Sigma Chemical Co., St. Louis, Missouri
[e] Draize: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965)

Mari McFarley 2/27/87
Sponsor Date

Gene L Harris 3/2/87
Study Director Date

Riker Experiment No. 0387MG0058

Appendix I (concluded)
Deviations and/or Amendments to Protocol

1. The induction phase will be limited to 6 doses. Reason for change: to avoid mortality in the study animals due to test material.

Gene L. Harris — 3/17/87
Study Director — Date

2. Pyridostigmine bromide may also be named "S-26741" in the raw data of this study. Reason for change: S-26741 is the Riker # given to pyridostigmine bromide.

Gene L. Harris — 4/14/87
Study Director — Date

3. _____

Study Director — Date

4. _____

Study Director — Date

APPENDIX II

Principal Participating Personnel Involved in the Study

Name	Function
G. L. Harris, B.S.	Advanced Toxicologist Study Director
G. E. Hart	Master Laboratory Technician Acute Toxicology
L. O. Wiseth	Sr. Laboratory Technician Acute Toxicology
J. A. Eads	Jr. Laboratory Technician Acute Toxicology
J. L. Allen, Ph.D.	Research Toxicologist Acute Toxicology
G. C. Pecore	Supervisor Animal Laboratory

APPENDIX III

Test and/or Control Article Characterization

for

Hydroxypropyl methylcellulose gel containing 30% pyridostigmine Brom. and 0.198% Sodium Lauryl Sulfate, (FN 4590)

1. The identity strength, uniformity, composition, purity or other pertinent characterizations of the test and/or substances have been determined and documented as of RFA 14202-2/27/87.

2. The method of synthesis or origin of the test and control substances, including their amount and the method of bioassay (if applicable) is documented.

☐ Yes ☐ No Hand written 2/27/87

3. The stability of the test and/or control substances ~~have been determined~~ * or will be determined ~~as of~~ at the end of the study.

Hand written 2/27/87

The above information and documentation are located in the sponsor's records.

~~Sponsor or~~ Sponsor Representative	Date
Hand written	4/29/87

Original characterization can be found in Exp# 0387MB0057.

* = Form change

Form 19793 - A - PWO

APPENDIX IV

QUALITY ASSURANCE STATEMENT

Acute Toxicology Laboratory Studies

Study No.: 0387MG0058

This short term study was audited by Compliance Audit and the final report examined against the raw data on April 21, 1987. The results of the audit were reported to the study director and to management on April 21, 1987.

In addition to the data audit, different significant phases for studies underway in the Acute Toxicology Laboratory are inspected on a recurring cycle, and the facilities are examined by Compliance Audit on a three month schedule.

D. M. Marboe, Jr.
Compliance Audit

4-21-87
Date

Pathology and Toxicology
Riker Laboratories, Inc.

Building 270-3S-05, 3M Center
St. Paul, Minnesota 55144-1000

COMPANY CONFIDENTIAL

3M

Cytotoxicity Test - Agar Overlay with Microporous Membranes Using L-929 Mouse Fibroblasts

Riker Experiment No: 1187MK0018

Conducted At: Pathology and Toxicology
Riker Laboratories, Inc.

Dates Conducted: January 21, 1987 to March 24, 1987

Conducted By: G. E. Hart 12/29/87
G. E. Hart Date
Acute Toxicity Study Coordinator
Study Director

Reviewed By: J. Allen 12-29-87
J. L. Allen, Ph.D. Date
Diplomate, A.B.T.
Research Toxicologist

dc: J. L. Allen
R. T. Catherall
S. V. Elrod
M. J. Westfall
Tech. Doc. Center
Path/Tox Files

Summary

Screening cytotoxicity tests were conducted from January 21, 1987 to March 24, 1987 at Riker Laboratories, Inc., St. Paul, Minnesota on microporous membranes used in pyridostigmine transdermal formulations. The data was used for selection of membranes for further studies.

The raw data is not available for these screens, but attached is a sample protocol which presents the methods used for the study.

Introduction

The objective of this study was to determine the response of a mammalian monolayer cell culture to any readily diffusible components of microporous membranes, using L-929 mouse fibroblasts. This study was conducted for research and development purposes and is, therefore, not regulated by the Food and Drug Administration's Good Laboratory Practice Regulation of 1978, although the standard operating procedures of this laboratory adhere to the general principles of this regulation.

Riker Experiment No. _____

PROTOCOL

TEST: Cytotoxicity Test — Agar Overlay

SPONSOR: 3M _____ Division

CONDUCTED BY: **Pathology and Toxicology Department, Riker Laboratories, Inc. St. Paul, Minnesota**

TEST ARTICLE: _____

CONTROL ARTICLE: _____

PROPOSED STARTING/COMPLETION DATE OF TEST: _____

TEST SYSTEM AND SOURCE: L-929 Mouse Lung Fibroblasts, CCl 1.2, ATCC Rockville, Maryland

OBJECTIVE. To determine the response of a mammalian monolayer cell culture to readily diffusible components from test articles. The L-929 cells are used as the test system due to their extensive characterization in the literature and their ease of maintenance.

METHOD. The method to be used is similar to that of Guess et al[a] . A confluent monolayer will be propagated in medium[b] in a 6-well petri dish. The medium will be aspirated and the agar, containing minimal nutrient requirements[c] of the cells and neutral red dye[d] , layered over the cells. The test article (_____) will be placed on the surface of the agar. Each petri dish will receive four test samples, one positive control[e] (_____) and one negative control[f] (_____). All samples will be identified on the lid of the petri dish. After application of the test samples, the petri dish will be incubated at 37°C overnight in a 5% CO_2 atmosphere. The response of the cell monolayer will be evaluated with respect to the extent of decolorization of the stained monolayer under and around the sample (zone) and the estimated extent of lysis of the cells within the decolorized zone. A sample will be reported as CYTOTOXIC only if lysis is observed.

Zone Index	Description of Zone
0	No detectable zone around or under sample
1	Zone limited to area under sample
2	Zone not greater than 0.5 cm in extension from sample
3	Zone not greater than 1.0 cm in extension from sample
4	Zone greater than 1 cm in extension from sample, but not involving the entire plate
5	Zone involving entire plate

Lysis Index	Description of Extent of Lysis (Microscopic)	CYTOTOXICITY RATING (Based on the average % lysis)	
0	No observable lysis	0.0	= Not Cytotoxic
1	Less than 20% of the zone lysed	0.1 - 0.9	= Minimal
2	Less than 40% of the zone lysed	1.0 - 1.8	= Mild
3	Less than 60% of the zone lysed	1.9 - 2.8	= Moderate
4	Less than 80% of the zone lysed	2.9 - 4.0	= Severe
5	Greater than 80% lysed within the zone	> 4.0	= Extreme

All raw data generated by the study director and the final report will be stored in the Riker Laboratories' Archive, St. Paul, Minnesota.

[a] Guess, W.L. et al, "Agar Diffusion Method for Toxicity Screening of Plastics on Cultured Cell Monolayers" J. Pharm. Sci. 54:1545-1547 (1965)

[b] Dulbecco's Modified Eagle Medium fortified with 10% fetal calf serum (HI) and 10 ml Pen (10,000 u/ml)/Strep. (10,000 mcg/ml), GIBCO, Grand Island, New York

[c] 2X Modified Eagle Medium, GIBCO, Grand Island, New York

[d] GIBCO, Grand Island, New York

[e] Plasticized PVC film: 67.9% PVC resin. Diamond Shamrock 426, Lot 95729
30.6% Dioctylphthalate
1.4% Diacetoxydibutyl tin
0.06% Stearic acid

[f] Bev-A-Line Thermoplastic Processes Inc., Stirling, New Jersey

Sponsor _____ Date _____ Study Director _____ Date _____